Socioeconomic Status and Health: Exploring Biological Pathways

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This thesis describes The Biomarkers of Social Disadvantage study. The initial concept was developed in collaboration with researchers from the National Centre for Epidemiology and Population Health and the Centre for Mental Health Research. Design of fieldwork protocols, data collection, entry and analysis and writing up are my original work.

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Robyn Lucas, BSc MB ChB MPH&TM
A Dedication

To my beloved father,

Jim Lucas,

who died September 24, 2003,
during the final stages of thesis preparation.
Acknowledgements

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Abstract

The cross-sectional Biomarkers Study was undertaken in Canberra, Australia (2000-2002) to examine the role of psychosocial factors in the socioeconomic health gradient, via physiological changes consequent upon activation of the neuroendocrine stress response.

The study population was derived from healthy 40-44 year old men and women already participating in a longitudinal cohort study. Using data from the cohort study, four groups with similar occupational status were formed. The study sample was randomly selected within these groups, thus representing the socioeconomic spectrum.

A pilot study involved 60 participants with blood and saliva samples measured on two occasions. A further 302 people had blood and saliva samples taken on one occasion. Socioeconomic status was measured by occupational code and status, personal and household income, education and perceived position in the community and in Australia. Psychosocial and behavioural factors, including job strain, job security, coping style, anxiety, depression, optimism, self-esteem, sense of belonging and trust, social support, smoking, exercise and alcohol intake were assessed by self-report. Five biological parameters: plasma fibrinogen, glycated haemoglobin, waist-hip ratio, serum neopterin and salivary IgA were measured as outcome variables. Three hypotheses were tested:
1. There is a socioeconomic gradient in measures of psychosocial stress, and of psychological resilience.

2. There is a socioeconomic gradient in biological measures that have a plausible association with future disease.

3. Psychosocial factors mediate the demonstrated association between socioeconomic status and the biological measures.

Data analysis confirmed a socioeconomic gradient in some psychosocial and behavioural variables: economic strain ($r=-0.44$, $p<0.001$), job demands ($r=0.45$, $p<0.001$), job control ($r=0.26$, $p<0.001$), active coping style ($r=0.28$, $p<0.001$), sense of optimism ($r=0.24$, $p<0.001$), social capital ($r=0.26$, $p<0.001$), job security ($r=0.17$, $p=0.002$), job marketability ($r=-0.16$, $p=0.005$), sense of belonging ($r=0.22$, $p<0.001$), number of adverse life events ($r=-0.13$, $p=0.01$) and positive interaction with family and friends ($r=0.20$, $p<0.001$), vigorous physical activity ($r=-0.16$, $p=0.002$), alcohol consumption ($r=0.30$, $p<0.001$) and smoking status ($r=-0.25$, $p<0.001$). There was no socioeconomic gradient in anxiety, depression, neuroticism, hostility, locus of control, self-esteem, perceived stress or mental health (SF-12). Four of the five biological markers varied with socioeconomic status: plasma fibrinogen (female (F): $r=-0.26$, $p=0.002$, male (M) $r=-0.08$, $p=0.30$), glycated haemoglobin (F: $r=-0.23$, $p=0.01$, M: $r=-0.11$, $p=0.17$), waist-hip ratio (F: $r=-0.19$, $p=0.03$, M: $r=-0.27$, $p<0.001$), serum neopterin (F: $r=-0.21$, $p=0.009$, M: $r=-0.04$, $p=0.56$), salivary IgA (F: $r=-0.07$, $p=0.38$, M: $r=0.004$, $p=0.97$). A more adverse biological profile was associated with lower socioeconomic status. Work characteristics, coping style, smoking and exercise were particularly important mediators of the association between the biological markers and socioeconomic status. Particular psychosocial
factors were consistent mediators of the association between specific biomarkers and socioeconomic status (with little variation for different measures of socioeconomic status). However, the particular psychosocial factors providing significant mediation varied for the different markers.

In this sample of healthy 40-44 year olds, four out of five biological markers showed moderate socioeconomic variation with a more favourable profile associated with higher SES. The data provide limited support for the importance of psychosocial factors in the socioeconomic health gradient.
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Glossary

**Acrophase**: The time of the day when an individual is typically at his/her highest level of the characteristic of interest.

**Acute phase response**: A physiological response to injury (and probably stressor exposure), mediated by cytokines and glucocorticoids, which results in the hepatic release of acute phase proteins (including fibrinogen, haptoglobin and C-reactive protein), as well as mediators of pain, leukocyte trafficking and vessel permeability.

**Adrenocorticotrophin hormone** (ACTH): A hormone released from the anterior pituitary gland under control of CRH and negative feedback control from cortisol levels. ACTH stimulates the adrenal cortex to produce glucocorticoids, of which cortisol is of primary importance in human physiology.

**Autonomic nervous system** (ANS): A subdivision of the motor nervous system of vertebrates that regulates the internal environment. It consists of parasympathetic and sympathetic divisions.

**Corticotrophin releasing hormone** (CRH): Produced primarily by the parvocellular neurons in the paraventricular nuclei of the hypothalamus and stimulating the anterior pituitary release of ACTH. There are also CRH-producing neurons in the paragigantocellular and parabranchial nuclei of the medulla and the locus coeruleus allowing communication between the fast and slow stress responses.
**Cortisol**: a steroid hormone released from the adrenal cortex. Cortisol has multiple effects on the metabolic system resulting in increased blood glucose and may suppress the immune system.

**Cytokines**: protein factors secreted by macrophages and helper T cells as regulators of neighbouring cells, e.g. tumour necrosis factor alpha (TNF-α), interleukin 6 (IL-6).

**Gini coefficient**: A quantitative measure of inequality, based on the Lorenz curve. The higher the Gini coefficient, the greater the level of inequality.

**Glucocorticoid receptor** (GCR): the cytoplasmic receptor to which glucocorticoids attach to exert their intranuclear action on target genes.

**Hypothalamic pituitary axis** (HPA): Incorporates the hypothalamus, hormone releasing factors, the anterior and posterior pituitary and the hormones released from these regions.

**Interleukins**: specific cytokines released by different T cells of the immune system. Currently interleukins are labelled 1-15, i.e. IL-1, IL-2 etc.

**Lymphocytes**: A family of white blood cells. Lymphocytes that complete their development in the bone marrow are called B cells, and those that mature in the
thymus are called T cells. T cells are further differentiated based on a cluster of
differentiation (CD) number, e.g. CD 4.

**Parasympathetic nervous system** (PNS): A division of the autonomic nervous
system, that enhances body activities that gain and conserve energy, such as digestion
and reduced heart rate.

**Sympathetic adrenal medullary** (SAM) system: The combination of the pathways
of the sympathetic nervous system and the adrenal medulla, activation of which
results in the release of adrenaline.

**Sympathetic nervous system** (SNS): A division of the autonomic nervous system
that generally increases energy expenditure and prepares the body for action.
Chapter 1.

Introduction

Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity. *Preamble to the Constitution, WHO, 1946* [1]

Everyone has the right to the enjoyment of the highest attainable standard of physical and mental health. *International Conference on Population and Development, Cairo 1994* [2]

1.1 Health and hierarchy

This thesis investigates aspects of health and socioeconomic inequality and how these interrelate. Beginning from a basis of socioeconomic difference, it examines variation in a number of psychosocial factors and how these might mediate socioeconomic variation in physiological measures that are plausible precursors of ill health. The research is nested within the broader framework of the socioeconomic determinants of health, which have been widely documented in most developed countries, including Australia. Briefly, research indicates that in most developed countries and for most health outcomes, there is a positive gradient in health in relation to socioeconomic status (SES), regardless of the health and SES measures used [3]. This is a consistent finding and notably, is not just a difference between the most deprived groups and all others. Rather, it is a finely grained association, present throughout the whole range of socioeconomic status [3]. This characteristic of the association is extremely important – at any point on the socioeconomic health gradient, people have
better health than those below them and worse health than those above. That is, poorer health outcomes are not only a result of absolute deprivation or poverty, but also of relative deprivation.

Difference is a basic attribute of nature. Genetic diversity, tempered or enhanced by the environment, has been responsible for the evolution of all life. Natural selection and “survival of the fittest” are inequality-based processes. Why did individuals of some species survive adversities such as ice ages, to ensure continuation of their species, when others died? Whether due to differences in physical features, survival following injury, or intelligence in choices, inequality has framed evolution. But humankind is special – for the first time a species is not primarily at the mercy of nature’s selective pressures, but rather, through the development of culture, has been able to subvert the process, mould nature to its own purposes and to flourish, even in apparently hostile environments, almost without restraint [4].

The physical evolution of animals has been accompanied by the evolution of societies, in which each individual has a place and a role. Bee societies contain workers, drones and a queen, each with different roles. In ant colonies there are workers, males and a queen, with some colonies having subdivisions of the worker caste, with different roles. Many higher animal species display social dominance hierarchies, in which the strongest assume leadership roles. Weaker or less able individuals adopt, or are forced to adopt, subordinate roles. Animal research indicates that such social hierarchies are also associated with hierarchies of health [5]. In a somewhat circular manner, the fittest are dominant and the leaders have the best
health. Those who are subordinate suffer poorer health and those with poorer health are subordinate [5, 6].

Modern human societies also tend to be characterized by dominance hierarchies, based on those attributes typically used to denote socioeconomic status – education, occupation, wealth and income. As in animal hierarchies, there is a health cost to being in a lower socioeconomic position. As Evans writes, “Top people live longer” [3]. Such socioeconomic variations in health have been observed over many centuries [7].

With the recognition, late in the nineteenth century, of the importance of infectious agents in disease causation, the biomedical model, including the belief that a specific agent caused each particular disease, was prominent in health research [4]. Humans were considered as being composed of interacting but separate “systems”, each available for study by different specialists. Physiologists studied biological processes, psychologists the mind, neurologists the nervous system, endocrinologists the endocrine system, immunologists the immune system and sociologists the social environment in which humans lived. In the last thirty years the threads from these and other disciplines have been brought together in hypotheses that emphasise the importance of the social environment as well as individual psychological attributes, drawing on new knowledge of the pathways through which social and psychological experiences are embodied and thus may affect health [3, 7].
While the biomedical model is a useful framework, it fails to span the breadth of influences on health. The social environment somehow affects individual psychology, physiology and even the immune system. In fact, each component sphere of influence has a bi-directional relationship with each other component and research has actively attempted to delineate the nature of these relationships. While much work remains to be done, it is clear that over the billions of years of evolution, complex systems have developed to deal with the complex challenges of a changing internal and external environment.

The current study aims to further inform the search for the routes by which health is associated with socioeconomic status. The literature distinguishes between fundamental, distal and proximal causes of health inequalities. Proximal causes are those immediately preceding diseases – smoking causes lung cancer. They typically act on individuals. Distal causes act via proximal causes to produce disease. One might question why people continue to smoke despite clear evidence that it causes lung cancer. The answer may be found in societal processes that induce teenagers to start smoking and produce a disparity in the ability to cease smoking later in life [8]. Fundamental causes are the root causes that are not themselves caused by something higher up the causal chain. Some would say that socioeconomic status is a fundamental cause of disease [9]. Certainly a hierarchical social structure is common in primate species (including Homo sapiens), and is associated with health disparities [6]. As a fundamental cause, socioeconomic status underlies the societal processes that are distal causes and these in turn determine the proximal causes of disease.
1.2 The social structure of disease

Several models have been constructed to depict the hierarchy of disease causation. Two are presented in Figures 1.1 and 1.2.

Each model emphasises the many layers involved in disease causation, in particular the importance of factors upstream of, or distal to, the individual. Social structure is depicted as a fundamental cause of health, while the social, community and work environments act through individual factors to affect the health of individuals, and thus cumulatively the health of populations.

Figure 1.1. The Dahlgren and Whitehead model of the source of health inequalities 1991(cited in [10])
Figure 1.2. An approach to sketching in the environmental, psychosocial, and biological pathways linking socioeconomic status to diabetes mellitus (DM), coronary heart disease (CHD), and well-being. Adapted from [11]

Marmot’s model (Figure 1.2) more explicitly addresses the routes by which distal factors are embodied in individual pathophysiological processes, with modulation by health behaviours and genetic heritage. But, perhaps necessarily, each model is greatly simplified with a lack of feedback arrows from the final health effects on the individual, back to the more distal determinants. In reality, there is a dynamic interaction between the social, community, and work situations and individual health and well-being.
1.3 The Biomarkers Study

This thesis examines the most proximal pathways – the end-point where proximal, distal and fundamental causes are translated by biological processes into biological aberrations and subsequent ill health. It is based on a psychosocial hypothesis of the causation of socioeconomic health inequalities – that psychosocial factors, working both distally and proximally, are somehow embodied and alter disease susceptibility. This is a study of healthy middle-aged individuals, the age at which, in many studies, health inequalities are most marked. In some studies it is difficult to be sure that the measured psychosocial factors preceded the disease, and are not a result of the disease process. The current study does not examine disease outcomes; rather it examines biological measures that are plausible associates of an early disease process. Measured on blood and saliva, there can be no question of reverse causation. Thus, although this is a cross-sectional study with temporal limitations in regard to causal inference, the use of biological markers overcomes the possibility of reverse causation and justifies the use of mediation analysis, which uses a causal framework.

Specifically, the thesis aims to investigate the following hypotheses:

1. There is a socioeconomic gradient in measures of psychosocial stress, and of psychological resilience.

2. There is a socioeconomic gradient in biological measures that have a plausible association with future disease.

3. Psychosocial factors mediate the demonstrated association between socioeconomic status and the biological measures.
The psychosocial hypothesis suggests that there is a differential distribution of personality characteristics and of psychosocial stress, such that those of lower socioeconomic status suffer more stress and have a lesser ability to manage this stress, through poorer coping styles, lower self-esteem etc. The bodily stress response, mediated by the neuroendocrine system, results in alterations to body physiology that may ultimately cause disease.

1.4 The study setting

The research is set in Canberra, the capital of Australia and its immediate surroundings, including Queanbeyan, a satellite town in neighbouring New South Wales. Canberra is a small city, with a population of 312,000 people, while Queanbeyan has a population of 31,000 [12]. The Australian Federal Government is a major employer in the region.

Australia has a system of universal medical insurance, Medicare, which provides subsidized medical care to all residents, regardless of age or circumstances [13]. Medicare pays a fixed rebate for service, but there is no legislation restricting the amount a doctor can charge for a service. Patients pay the gap between the fee set by Medicare and that charged by the doctor. Most general medical visits in the region entail an out-of-pocket cost of at least $10-$20. Heavy users, or those on government welfare programmes, have access to a health care card that allows them free medical care. Young and colleagues studied use of medical services in Australia and found that lower SES was associated with lower out-of-pocket costs, suggesting that there is
some adjustment by the health care system for income inequity [13]. Australia-wide, 30.5% of the population has private health insurance in addition to Medicare.

Compared to the rest of Australia, Canberra has an affluent population – indeed, in the 2001 census, the Australian Capital Territory (ACT) had the highest median weekly individual income (for people aged 15 years and over) of any state or territory in Australia ($500-599, compared to $300-$399 for Australia). It is a young city, having just celebrated its 90th birthday, and carefully planned – the result of an architectural competition. The plan includes designated open space, delineated suburbs each with a local shopping centre, arterial bus routes for public transport and a mix of housing types in most suburbs. The Canberra climate is hot in summer, with an average temperature of 27ºC, but with frequent days over 30ºC and occasional days over 40ºC. In winter, the average temperature is 11ºC, but with overnight temperatures commonly below freezing. Houses use a range of heating methods, including wood fires, freestanding electric and gas heaters and central heating. Smoking is banned in most indoor workplaces, in most restaurants and in public transport. Such community characteristics are important in determining access to health care, exposure to noxious environmental factors (such as smoke from wood fires and temperature extremes) and favourability of the environment for positive behavioural activities (such as exercise and not smoking). In addition, the forced mix of housing within suburbs may have implications for social cohesion within neighbourhoods.
1.5 Thesis outline

I begin the thesis with a review of the health inequalities literature (Chapter 2). Following an examination of the wider literature on the types of inequalities in health in the modern world, the chapter moves on to describe the nature and the causes of the socioeconomic variation in health. Several competing (and overlapping or complementary) hypotheses have been developed to explain the socioeconomic determinants of health, and the supporting evidence for these is explored. The review then focuses on the hypothesis that psychosocial factors are important in the causation of socioeconomic health inequalities, as this hypothesis forms the basis of the thesis. From there, I review the rationale for the study and identify the contribution that this project can make to furthering our understanding of this important area.

The study investigates the routes by which socioeconomic status “gets under the skin” to affect health. As such it has a strong biological emphasis. In order to understand how this biological embedding may operate, in Chapter 3, I outline the physiology that underpins the psychosocial hypothesis wherein wide-ranging effects on multiple body systems cause physiological alterations, mediated by the neuroendocrine system. This leads to an examination of the rationale for using biological outcome variables and the requirements of an ideal marker. Finally, I outline the measures chosen and review each of these in some detail to show how they plausibly link to both the psychosocial hypothesis and to disease outcomes.

Chapter 4 outlines the methodology used in the Biomarkers Study. Several measured and derived aspects of SES were examined and their measurement is outlined. I detail
the mechanics of data collection and the analysis of the biological samples. This investigation was undertaken as a sub-study of the Personality and Total Health (PATH) Study, which is briefly described. The origin and derivation of the psychosocial measures is outlined. Procedures for data collection, recording, manipulation and analysis are presented as well as information on the response rates and the likely representativeness of the study sample. The study limitations are discussed, as are sources of error and bias.

The results of the statistical analysis are presented in Chapter 5. This includes an examination of the correlation between different measures of SES (including childhood SES), as well as the variation of biological and psychosocial factors by different measures of SES. The chapter presents the results of the mediation analysis for each biological marker in which psychosocial, behavioural and biological factors are tested as mediators of the association between each marker and each measure of SES.

Chapter 6 presents the discussion of these results, within the context of the wider research knowledge. The value of each biological marker as an outcome variable for this type of research is examined. In particular the results are discussed within the framework of the psychosocial hypothesis with an assessment of the level of support that this research provides, for the hypothesis. The Biomarkers Study presents an assessment of the socioeconomic variation of a wider variety of psychosocial factors than has previously been undertaken. Such variation is a critical part of the psychosocial hypothesis, and the study explicitly examines the variation of not just
psychosocial stressor exposure, but of elements of psychosocial resilience. By using healthy individuals, and biological measures as the outcome variables, the study avoids the problem of reverse causation, while using middle-aged individuals aims to measure the age group for which socioeconomic health inequalities have been greatest in previous research.

Chapter 7 concludes the dissertation with a brief summary of the findings and suggestions for future research directions. While this thesis is based on the examination of associations in individuals, the biological translation of external experiences is recognised as a final pathway by which wider political, social and economic forces influence the health of individuals and populations. The population-level implications of the current research and of the psychosocial hypothesis are discussed.

In the last thirty years there has been an increase in interest, and significant progress made, in describing and understanding the socioeconomic determinants of health. Distinct groups of researchers have proposed and championed particular hypotheses of causation and often presented these as competing and exclusive theories. They are, I believe, not competitive but complementary, and different aspects of the same complex story, with a final common pathway via psychosocial effects, indirectly on proximal behavioural risk factors and directly via the neuroendocrine stress response and subsequent physiological change. This thesis examines this final common pathway, to provide supporting evidence for its existence and importance.
Chapter 2.

Literature Review

2.1 Introduction

Health is central to the continuity of human life. To some extent it is determined by genetic heritage. But environmental influences moderate the expression of that heritage and the net effect is an unequal distribution of health and disease.

Research and thought from many disciplines permeates the health inequality literature – from physiology and medicine, to sociology, psychology, and economics. This chapter briefly spans the breadth of this research with a gradually narrowing focus, culminating in the identification of my specific research questions.

I begin with some general comments about inequalities in health and then focus on socioeconomic inequalities in health. After considering the global picture, I review the international literature and the situation in Australia. I explore the various theories of causation for this phenomenon and then focus on the psychosocial hypothesis in some depth. The following sections narrow the focus further to explore the rationale for looking for markers of health disparities due to socioeconomic status, the qualities that would be desirable in such markers and then the logical possibilities from current knowledge of physiology. I finish the chapter by identifying three research questions, aimed at clarifying aspects of the psychosocial explanation for socioeconomic health inequalities.
2.2 Health inequalities

Health is not a resource that is equally shared among different groups in society, in any society. Women live longer than men. Minority groups, and particularly indigenous populations who are minority groups, fare badly compared to dominant groups. And the wealthy live longer and suffer less ill health than those who are less wealthy.

2.2.1 Gender

There are gender inequalities in health. On average, women live longer, make more use of health services but may experience greater morbidity, than men. “For every age group, male mortality is higher than that of females, life expectancy is lower for men, men tend to use primary health services less than women, are more likely to delay help-seeking when ill and are more likely to adopt health damaging or ‘risky’ behaviours” [14]. Research indicates that the determinants of health may be different for men and women. Denton suggests that for women, social structural factors, such as being in the highest income category, working full-time and caring for a family and having adequate social support are more important predictors of good health than for men. The relative importance of various behavioural determinants of health may also vary by gender, with smoking and alcohol consumption more important for men’s health, but body weight and physical inactivity more important for women’s health [15].

Some research suggests that ‘gender identity’ has a role in health differences between men and women. Health is seen as women’s business and responsibility, rather than
men’s; men tend to keep quiet about their health problems and know little about men’s health; health and health promotion is ‘female’ and so being a man means being denied a self-monitoring role; men delay seeking help and tend to see the body as a machine, so that there is an allowance that they don’t deal well with emotions and feelings [14].

In addition, socioeconomic gradients in health may vary by gender. This may, in part, depend on the unit of measurement used for socioeconomic status [16] and at least for occupational measures may be associated with the differential employment rates and types of employment available to men and women [17]. There is likely to be a complex interaction between SES, marital status, work and home responsibilities, and gender, which affects health. Indeed, over the last forty years there have been such marked changes in the employment of women (at least in developed countries) that older studies examining gender differences in SES gradients may no longer be pertinent.

Gender differences in health probably result, not just from biological differences, but also from social and psychological influences, at least for morbidity. Gender may modify the effect of SES on health, with some studies showing that for diseases such as myocardial infarction, there is a steeper SES gradient for women than men [18], while for other diseases, the gradient is less steep, e.g. limiting long standing illness [19]. A recent review notes that the gender differential in the SES gradient for mortality is apparent only for absolute risk and disappears when the comparison is
made using relative risk of mortality [20], suggesting that pathways producing SES mortality gradients are similar for men and women.

2.2.2 Ethnicity

Minority groups and particularly indigenous populations who are minority groups, tend to have poor health compared to the rest of the population. In addition, in many countries indigenous people disproportionately occupy lower socioeconomic groups. For example, in New Zealand only 13% of Maori earn $30,000 or more, compared to the New Zealand average of 21.6%, and only 2.6% of Maori achieved a tertiary degree in 1996/7, compared to the NZ average of 8%. However, even when SES is taken into account, Maori still have worse health than non-Maori [21].

In the 2001 Census, 2.4% of the Australian population identified themselves as Indigenous Australians. On average, Indigenous Australians are much poorer than non-Indigenous Australians and experience worse health status on every indicator [22]. Life expectancy at birth in 1997-99 in Australia was 82.1 years for Australian women and 76.6 years for Australian men. We can compare this to the life expectancy at birth for Indigenous Australians: 63 years for women and 56 years for men [23]. And, while mortality rates are decreasing in Indigenous Australian populations, the gap in health between Indigenous and non-Indigenous populations is not declining [24]. Indeed this gap remains greater in Australia than in other similar countries such as New Zealand and Canada [25].
In addition to poorer education, lower levels of home ownership, lower incomes and higher rates of unemployment, Indigenous Australians are more likely to be classified as obese and are about twice as likely to smoke as non-Indigenous Australians [24]. But these factors do not fully explain the difference in health between population groups. Recent research in Indigenous Australian populations highlights the importance of the social environment, including the immediate local environment, friends, family and community and the perception of mastery or control, both in the workplace and the wider society, to health (Trudgen, 2000, cited in [24]).

In Canada, the average life expectancy of Registered Indians is approximately six years less than the overall Canadian population (Canada 1998, cited in [26]). This represents an improvement since 1990, when the life expectancy of male and female Registered Indians was 66.9 and 74.0 years respectively, compared to the total male and female Canadian population of 73.9 and 80.5 years respectively (Canada, 1996a cited in [26]).

What causes the poorer health of indigenous populations, over and above that related to SES and to the higher prevalence of health damaging behaviours, and can these causes inform research on disparities that are related to SES?

Indigenous populations vary significantly in culture and traditions, but most view health much more holistically than in western culture [21]. “Aboriginal people from almost every culture believe that health is a matter of balance and harmony within the self and with others, sustained and ordered by spiritual law and the bounty of Mother
Earth” (Royal Commission on Aboriginal Peoples Volume 3, cited in [26]). Health includes not only physical health but also social and emotional well-being, spirituality and the well-being of family and community groups [27]. The apparently all-encompassing definition of health adopted by the World Health Organization (see Introduction), appears narrow and individualistic in comparison.

The origins of ethnic disparities in health may include genetic, cultural or socioeconomic causes. But, in addition to questions of access to services, and material explanations, experiences of racism may be important determinants of ethnic health inequalities [28].

### 2.3 Health and Socioeconomic status

#### 2.3.1 Introduction

For countries with a gross national product (GNP) per capita of less than about $5000, there is a clear relationship between GNP and life expectancy. The relationship is quite steep, with small increases in per capita income related to large increases in life expectancy [29]. See Figure 2.1.

This is hardly surprising. At low levels of GNP per capita the driving forces of maternal, infant and childhood deaths are malnutrition and infectious diseases. As GNP increases, living conditions improve with resultant improvements in life expectancy.
So it has been over history. As developed countries have grown economically, and have undergone social modernization, public health initiatives have dealt with the hazards related to the physical environment – clean water, sanitation, nutrition and immunization, and this has been reflected in increases in life expectancy. But beyond about $5000 per capita, the relationship between GNP and life expectancy weakens; countries with the highest GNP are not necessarily those with the greatest life expectancy.

**Figure 2.1. Life expectancy and income for selected countries and periods (World Bank, 1993, cited in [29])**
Within countries, the shape of the curve linking socioeconomic status to health is remarkably similar to that between countries, i.e. the greatest gains in health are made from the lowest levels to the middle levels of SES, while the curve flattens with higher SES [30]. This relationship holds for most measures of socioeconomic status and for many health outcomes.

2.3.2 Historical Background

Observations of the presence of socioeconomic inequalities in health are not new. Antonovsky notes that lower life expectancy and higher death rates in the lower classes have been observed since the 12th century, when data on this question were first organized [31]. In 1848 Virchow, recognized that socioeconomic position was an important determinant of the factors driving infectious epidemics of typhus and other illnesses [32]. William Farr, in 1885, wrote, “the lifetime of sovereigns and peers is prolonged” [33]. At that time “the mean lifetime in the healthiest districts of England – and in the healthiest ranks – is 49 years; and we have no evidence that under the most favourable conditions it exceeds 50 years.”

During the late 19th century and the first half of the 20th century, the study of health became focused on the individual, with research centred on individual risk factors for disease. But in the 1960s there was a resurgence of interest in the issues of socioeconomic and racial inequalities in health, although it was thought that this was mainly caused by disparities in access to health care [32].
Meanwhile, in the early part of the 20th century, interest increased in the social production of health, beginning perhaps with Donnison’s book “Civilisation and Disease” in 1938, which theorised that the interaction of an individual with his/her social environment was a principle cause of disease. In 1949, Halliday’s book, “Psychosocial Medicine: A Study of the Sick Society” built on Donnison’s theory, with extension to a wider range of disease outcomes and causative factors, including urbanization, increasing instability of society and occupational exposures (cited in [5]). In 1974, Lalonde advanced the health field concept which “linked the macro structures of the social and physical environment……with the micro factors of lifestyle and biology” [34]. For the first time, it was proposed that access to health care, particularly physician and hospital care, was not a principle determinant of health disparities.

Psychologists postulated that emotion was a driving force in the evolutionary development of human biology [5]. The intrinsic rewards, via the limbic system, of species-preservative and self-preservative activities acted to reinforce these activities to ensure survival. The neuroendocrine components of emotion were beginning to be researched and understood.

Physiologists such as Cannon and Selye began to elucidate the neuroendocrine pathways involved in stress and distress in the early to mid 20th century. And in 1953, Wolff’s book “Stress and Disease” showed that laboratory induced stress could cause adverse changes in almost any human body system [5]. Holmes and Rahe extended this work to show that disease could also be associated with stressful life events [35].
In the mid-1970s Cassel proposed that not only psychological stress, but also the social environment, including dominance hierarchies, marginal social status and lack of social support, worked via neuroendocrine mechanisms to alter host susceptibility to a wide range of diseases [36]. Susser et al (1985) suggested that individuals of lower social status experienced more stress-related alterations of physiology in response to any particular stressor, as a result of poorer coping abilities (cited in [34]). Berkman and Syme found a link between having fewer social ties and an increased risk of mortality over nine years of follow-up [37]. There was both theoretical and empirical support for the importance of psychological and social factors in determining health.

The release of the findings of the first Whitehall study of British civil servants, in 1978 was a watershed in the development of research into health inequalities [38]. Previous explanations of the socioeconomic gradient in health had focused on poor health in those in the lower classes, with explanations including poor housing, crowding, poor education and unemployment, resulting in poor nutrition, poor medical care and increased exposure to noxious agents [7]. But in the Whitehall studies, Marmot described not a difference in health outcomes between the highest and lowest SES groups, but a gradient of health such that at any point on the SES-health curve, those just above have better health and those just below have worse health, in an employed population with good access to medical care.
In the last twenty years, there has been an explosion of interest, research and writing on the socioeconomic health gradient. Advances in different disciplines, as outlined above, have been drawn together to create theories of causation. Many of these have been extensively researched and vigorously debated in the literature. The following sections briefly review some of the recent research, the theories generated from it and the evidence supporting them.

### 2.3.3 Cross-national studies

In 1979, Rodgers published the first study that suggested a link, not only between income and health at the individual level, but between income inequality and health at the aggregated level of states and countries [39]. Examining cross-sectional data from 56 countries relating income and income distribution to health outcomes (including life expectancy at birth and at five years and infant mortality), Rodgers found that the most consistently significant association was not between absolute income and health outcomes, but between income distribution and health outcomes. Those countries with greater income inequality had higher infant mortality and lower life expectancy than more egalitarian countries.

Wilkinson has continued and extended this line of research. In 1992, he examined the association between income distribution and health in 23 OECD countries [40]. There was a low correlation between life expectancy and GNP per capita. In addition, the correlation between increases in GNP per capita and increases in life expectancy (over the sixteen years of 1970/71 to 1986/87) was very low ($r = 0.07$). Although data on income distribution were available for only nine countries, the results were
striking: three quarters of the variance in life expectancy was explained by GNP per capita and the proportion of income going to those below the seventh decile of income for each country. But of the variance explained, less than 10% was attributable to GNP per capita. Wilkinson also examined longitudinal data, which showed a high correlation between changes in income distribution and changes in life expectancy \((r = 0.80, p<0.05)\). Although these findings were based on a small and select group of countries, Wilkinson concluded that, “Overall, there is clear evidence of a strong relation between a society’s income distribution and the average life expectancy of its population.”

Similar results were found in a cross-sectional comparison of US states with respect to income distribution and mortality (strong association between increased income inequality and mortality \((r = 0.54, p<0.05)\) even after adjustment for poverty) [41]. Greater income inequality was also associated with higher rates of homicide and violent crime, higher rates of unemployment, poor social cohesion and a smaller proportion of total spending on education [42]. Kawachi et al went on to investigate and theorise the causes of these associations, concluding “A large gap between rich people and poor people leads to higher mortality through the breakdown of social cohesion” [43].

A number of studies internationally have supported the income inequality finding. For example, a longitudinal study of 21 regions in Taiwan reported that income inequality replaced absolute median income as the best predictor of mortality, during the course of economic growth [44]. Hales et al (1999) showed that income distribution had a
stronger association with infant mortality than GNP per capita, except in poorer countries [45].

These studies have led to the elaboration of the hypothesis that relative, rather than absolute, income is important to the health of individuals. More egalitarian countries have greater life expectancy and lower mortality and morbidity – the theory suggests that psychosocial pathways may be important, with individuals in less egalitarian countries perceiving themselves as relatively deprived and developing poorer health through the direct effects of chronic mental and emotional stress and the indirect effects of health-damaging behaviours [46].

However, while both the relative income hypothesis, and the role of social cohesion or social capital in explaining the importance of relative income to health have gained a considerable following, not all studies support the importance of income inequality to health. Studies with similar methodologies have produced conflicting results and there has been criticism of the interpretation of the results of Wilkinson’s original study.

Comparison of provinces within Canada indicated that income inequality was not significantly associated with mortality after adjustment for median household income [47], while within Denmark, parish income inequality was not associated with mortality, but individual household income was [48]. In their study of small areas in the United States, Fiscella and Franks found that community level income inequality
showed a significant association with subsequent community mortality, but that this effect was lost when adjustment was made for individual household income [49].

More recently, Lynch et al have re-examined the association between income inequality and health, using data from the Luxembourg Income Study. When data from the nine countries of the 1992 Wilkinson paper were examined, there was a statistically significant association between income inequality and life expectancy in 1989-92 (r = -0.45) (compared to r = 0.86 between more equal income distribution and life expectancy in the 1992 paper [40]. However, when a further seven countries were added to the analysis, the association disappeared (r = -0.09) [50]. Indeed, the graphs presented in this paper suggest that the negative correlation between the Gini coefficient and life expectancy at birth is driven largely by data from the United States (USA) and the United Kingdom (UK).

Studies examining the links between income inequality and health in the US states (which have provided the strongest evidence for the relative income hypothesis and the role of social capital) have also been repeated. Statistical adjustment for ethnicity [51] or for education [52] has been separately shown to account for all of the association between income inequality and health. However, other studies continue to confirm the differences in health outcomes for US states by their level of income inequality [53] even after such statistical adjustment. Even after controlling for personal characteristics and household income, individuals living in states with the highest levels of income inequality were 30% more likely to report their health as fair or poor than individuals living in more egalitarian states. Such effects were more
marked among those on low incomes (<$20,000 per year) than those on middle incomes (20% higher than more equal states) or high incomes (no effect).

Ecological studies compare populations and taking account of individual level variables adequately is difficult. Gravelle postulated that the relationship of income inequality to health is a statistical artefact resulting from the shape of the curve relating individual income to health [54]. Studies suggest that health is approximately linearly related to the logarithm of income in all except very high and low incomes (thus increasing income is associated with better health, but the returns diminish at higher levels of income) [55]. Thus in more unequal societies, transferring income from the poor to the rich will result in greater losses of health in the poor than gains in health by the rich. When income inequality increases, overall population mortality will rise, purely because there are more people who are poor – their own mortality risk depends on their own income without a need to postulate any direct effect of income inequality itself [54]. However, recent simulation studies have failed to support Gravelle’s artefact hypothesis and confirm the importance of income inequality, independent of personal income [30].

It is difficult to reconcile such conflicting findings. Statistical artefact probably does not explain the association between income inequality within countries, and their health outcomes. Wilkinson suggests that an effect of income distribution is not seen when small areas are examined, since small areas tend to be relatively homogeneous with respect to income [56]. But cross-national studies have also delivered conflicting results.
Pearce suggests that there may be an association between income inequality and health, but that this is not automatic [51]. Rather it depends on the social, political and cultural environment in which income differences are experienced and is enhanced in those countries that also systematically under-invest in safety nets for people in unfavorable circumstances [47, 51, 57, 58]. The association may be diminished by policies that ensure access to primary medical care [59] and education [52]. Coburn proposes that the decline of the welfare state is central; it is not so much income inequality that creates poor social cohesion and ill health, but neo-liberalism that creates all three [60].

2.3.4 Country-level studies

One of the first epidemiological studies to examine the socioeconomic health differential was published in 1973, by Kitigawa and Hauser. Using data from the 1960 Matched Records Study and the Chicago Area Study, they found a strong inverse relationship between education (years of schooling) and mortality for four race and sex classes, in individuals aged 25-64, but not in those over the age of 65. A similar relationship was found when income was used as the socioeconomic measure, but multivariate analysis suggested that the education and income were independently associated with mortality, rather than alternative measures of a single construct [61].

The Black Report, released in 1980, was commissioned by the British Labour Government, to assess the evidence on health inequalities in the United Kingdom and provide policy recommendations. Using an occupational class measure of
socioeconomic status, the report showed that in 1971, members of the lowest class experienced a mortality rate more than twice as high as members of the highest class, even after controlling for age and sex. In addition the report found that health inequalities had increased over the preceding 30 years, despite the introduction of the National Health Service, which was intended to provide equal access to health care for all [62].

As well as describing the presence of health inequalities, the report outlined four possible explanations for the socioeconomic health disparity:

- Artefact.
- Natural/social selection.
- Materialist/structural explanation
- Cultural/behavioural explanation

I return to these in more detail in a subsequent section (section 2.4), but by outlining these possible explanations for socioeconomic health inequalities, the Black Report supplied a framework for subsequent research.

More recently the Acheson report has reviewed the progress made since the Black Report in the reduction of health inequalities in the UK [63]. Health inequalities persist - the life expectancy of a baby boy with parents in the professional or managerial groups is estimated to be about 5 years more than one born to parents in partly skilled or unskilled occupations; life expectancy at age 65 is 2.6 years greater for men and 2 years greater for women, from the professional and managerial groups than for those who are partly skilled or unskilled. The report emphasises the breadth
of reform that is needed to reduce socioeconomic health inequalities, with a focus not only on proximal, but also on the fundamental and distal determinants of health [63].

Inequalities in health associated with socioeconomic status have been found in most countries that have been studied, for a variety of different health outcomes. In the Netherlands, Boshuizen demonstrated that Dutch men with higher education have 12 years longer healthy life expectancy than men with lower education [64]. In Japan, lower individual income was strongly associated with poorer self-rated health [65]. In New Zealand socioeconomic differentials exist for most health outcomes with lower life expectancy at birth, more cancer deaths from smoking, poorer self-rated health and higher disability rates in those of lower SES as defined by the NZ Deprivation Index [21].

SES gradients are seen for a wide range of diseases, including various cancers [66], cardiovascular disease [38], diabetes [67] and respiratory diseases [68] with lower SES associated with higher rates of disease. For a few diseases, there is a positive SES gradient, e.g. breast, pancreatic, kidney, thyroid and colon cancer [69], while for others there is no apparent SES gradient, e.g. cancer of the rectum, prostate, and bladder and cancers of the haemopoietic system [69].

Socioeconomic inequalities in health are not just an issue in affluent countries. In Sao Paulo, Brazil during the late 1980s, adults in non-professional jobs had death rates two to three times higher than those of professionals; and in Venezuela, poorer municipalities have had infant mortality rates three times higher than those in other
municipalities [70]. Indeed, health disparities appear to be widening in low and middle-income countries, as well as in high-income countries [70].

Population health status is very malleable, over short time spans that rule out effects of changes in genetic endowment as the explanation [3]. Rather, health differentials appear to alter rapidly with changing social and political circumstances, even for diseases whose etiology is based on chronic physiological change e.g., coronary heart disease. In Russia, between 1990 and 1994, life expectancy fell from 63.8 to 57.6 years among men and from 74.4 to 71.0 years among women. This was a time of political upheaval and rising income inequality. Heightened violence and increased levels of alcoholism may have driven this change, but it is difficult to separate these factors from the direct or indirect effects of income inequality, decreased safety nets and/or decreased social cohesion as a result of the social and political instability [70, 71].

Similarly rapid change is evident in the comparison of life expectancy between Australia and New Zealand. Prior to 1970, Australia and New Zealand had similar trends in life expectancy with a fairly flat curve over time. In the early 1970s life expectancy in Australia began to increase faster than in New Zealand and this trend persisted for about ten years. Since the early 1980s the two countries have had virtually parallel increases in life expectancy, with Australia ahead by one to one and a half years. While many causes could be postulated for this difference, the timing parallels reductions in welfare funding in NZ in the mid-1970s [72].
In the last fifty years in Japan, there have been dramatic improvements in mortality rates, which have gone from being markedly worse than most European countries to markedly superior. This improvement does not appear to be due to increased health expenditure or beneficial lifestyle changes, although there have been changes in diet (more meat protein) and class structure, with a substantial reduction in the proportion of self-employed farmers and an increase in the working and middle classes [73]. Evans postulates that the improvement in health status may be driven by a change in the hierarchical position of Japanese society as a whole, relative to the rest of the world [3].

2.3.5 Individual-level studies

In 1978, Marmot published the first results of the Whitehall study looking at employed British civil servants. This was a longitudinal study in which mortality over several years was related to occupational grade. Marmot et al found that mortality from coronary heart disease differed more than three to one from the lowest to the highest grades of the civil service [38]. Much of this difference remained unexplained after adjustment for traditional risk factors for heart disease – smoking, serum cholesterol, blood pressure, glucose tolerance and obesity. Importantly, Marmot showed that the difference in health was not simply due to poor health in those in poverty – all participants were employed civil servants. Rather this was a gradient in health, in which at any point, those higher up the socioeconomic scale experienced better health than those below them and worse health than those above them.
The second Whitehall study sought explanations for the gradient, in particular that seen in coronary heart disease incidence and mortality [74]. In more than 11,000 civil servants, adjustment for the traditional cardiovascular disease risk factors (smoking, blood pressure, serum cholesterol, age and glucose intolerance) explained less than half of the socioeconomic gradient in the incidence of coronary heart disease. A measure of workplace job control explained most of the remaining variance [75].

Other studies examining the causes of SES health differentials have shown mixed results. Some have confirmed the findings from the Whitehall II study that traditional risk factors for disease fail to fully explain the SES gradient in health [76]. However, a recent study found that fourteen measured risk factors almost fully accounted for the SES health differential [77]. Social status was measured only by housing tenure (renter or owner-occupier), which is a relatively coarse measure of SES. In addition, one of the risk factors was plasma fibrinogen, which itself has been shown to be elevated with psychosocial job stress [78]. One difficulty lies in determining how factors should be entered into explanatory models of the effects of SES on health. Adjusting for proximal risk factors such as smoking, physical activity, fibrinogen, job stress etc may leave no residual SES effect, but these risk factors may be on the causal pathway between SES and health. Such proximal risk factors may mediate, rather than confound the SES/health association, i.e. they represent a route from fundamental and distal risk factors, to disease.

It is not just physical health that varies with socioeconomic status. In a cross-sectional study of adults in the US, there was a strong gradient between lower income and
symptoms of depression [67]. Lower income women living in a state with high income inequality, were particularly likely to report depressive symptoms [79].

Subclinical disease also shows a socioeconomic gradient, with asymptomatic men and women in their 30’s in lower social classes (as assessed by occupation and education) having significantly higher prevalence of coronary artery calcification on computed tomography [80]. In Whitehall II, heat shock protein (HSP60) was measured as a marker of CHD susceptibility in 229 adults, 45-58 years old. There was a significant inverse association between HSP and socioeconomic status [81].

Although SES inequalities in health are seen at all ages, the greatest disparities occur in middle adulthood [82].

### 2.3.6 Australian Studies

In a recent review of Australian research into the association of health and socioeconomic status, Turrell and Oldenburg state,

“Taken as a whole, the evidence on SES and health in Australia is unequivocal: those who occupy positions at lower levels of the socioeconomic hierarchy fare significantly worse in terms of their health. Specifically, persons variously classified as ‘low’ SES have higher mortality rates for most major causes of death, their morbidity profile indicates that they experience more ill-health (both physiological and psychological), and their use of health care services suggests that they are less likely to act to prevent disease or detect it at an asymptomatic stage. Moreover, socioeconomic differences in health are evident
for both females and males at every stage of the life-course (birth, infancy, childhood and adolescence, and adulthood) and the relationship exists irrespective of how SES and health are measured” [83].

More than three decades of research has confirmed the presence of significant inequalities in health in Australia, with those in lower SES groups having poorer health and higher mortality than those economically better off. Early research in the 1970s and 1980s reported increased all-cause mortality rates in lower SES groups as defined by occupational status [84] or occupational group [85]. Middle-aged men and women suffered an increased probability of chronic illness (both physical and mental) associated with lower income, education [86] and occupation [87]. Although mortality rates from ischaemic heart disease decreased by 25% from 1969 to 1978, the greatest decline was in upper SES groups [88].

While these early studies largely described the association of health with socioeconomic status, some researchers were already beginning to challenge the causes of this health gradient. McMichael (1981) stressed “the co-existent influences of work environment, socio-cultural environment, and personal behaviour and psychological characteristics” in the determination of health [85, 89]. Others tackled methodological issues - Najman critically reviewed methods of measurement of socioeconomic status for use in health related research [90] as well as noting the relative lack of participation in this research of those most severely disadvantaged [91].
Family composition and marital status were recognized to be important determinants of health, particularly in association with socioeconomic disadvantage. Never married, divorced or widowed males of lower occupational status were found to have higher mortality rates than other groups [92], while men who had never been married had a standardised death rate 124% higher than married men. In a study of social participation, Baum found that people with lower incomes were more likely to visit their neighbours, but were less likely to visit or be visited by family than those with higher incomes. However, there was no correlation between physical health and informal social contact, though those with poorer mental health and high social isolation reported less informal social interaction [93].

In 1992, the National Health Strategy published “Enough to make you sick. How income and environment affect health”. In this comprehensive summary of the state of Australia’s health with respect to SES, the authors noted that those of lower SES were more likely to suffer disability, have serious chronic illnesses, suffer recent illnesses and to report being in only fair or poor health (as opposed to good or excellent health). Similarly the most economically disadvantaged made the most use of primary and secondary health services, but were the lowest users of preventive services. A number of behavioural risks were more common in those of lower SES, including smoking, poor diet and physical inactivity. In addition the importance of social support was noted [94].

More recently there has been considerable research using ecological measures of socioeconomic disadvantage to characterize areas. The Australian Bureau of Statistics
has developed the Socioeconomic Indexes for Areas (SEIFA indexes) in which the socioeconomic aspects of areas are summarized using principal component analysis of census data [95]. There are five indices, of which the most used in health research has been Index of Relative Disadvantage. Using the SEIFA index it is apparent that while mortality rates declined between 1985-7 and 1995-7, SES differentials persisted and in some instances increased. For example, among males aged 25-64 years there has been an increase in mortality inequality for diseases of the circulatory system (including coronary heart disease), cancer (including lung cancer) and asthma/emphysema. For females, mortality inequality has increased for coronary heart disease, diabetes mellitus, cancer and respiratory disease. These patterns have tended to be in the form of a differential decrease in mortality for those in more advantaged areas rather than an increase in less advantaged areas [68].

Area effects on health (as opposed to the effects of personal SES on health) have been investigated, with research examining the health of individuals in similar occupations, in different areas within the same state. Dobson et al found that mortality rates from ischaemic heart disease in different areas of NSW did indeed show the usual SES gradient, but that rates were higher, for most occupational groups, in the Hunter Valley compared with the rest of New South Wales. Particular features of this region such as social capital, income inequality etc were not examined [96].

Health behaviours associated with socioeconomic status have been well studied, such as smoking [83, 97] and food consumption patterns [98]. While the expected trends in smoking prevalence were demonstrated, with increasing prevalence in lower SES
groups as defined by education and occupation, there has been a lack of consistent evidence that food consumption patterns vary by SES.

Is income inequality increasing in Australia?

During the 1990s, income inequality nationally increased significantly, as evidenced by an increase in the Gini coefficient (see Glossary) from 0.311 in 1990 to 0.328 in 1999-2000, with an upward trend throughout the decade. Similarly, those in the top ten percent of the income distribution increased their share of total income from 22.7% in 1990 to 23.9% in 1997-8. While those in the bottom ten percent maintained their share of the total income at around 2.5%, the middle 20 percent experienced a decline from 17.8% to 17.3% over the same period. Through the 1990s there was increasing spatial income inequality, with affluent areas tending to become richer and middle and lower order areas becoming less affluent. Overall, while there was little change in the relative socioeconomic positions of middle and lower SES groups, higher SES groups tended to become more distant from the other groups [99].

2.4 Theories of causation

The Black report outlined four possible causes of socioeconomic health inequalities. As listed above, these are:

- Artefact
- Natural/social selection
- Materialist/structural factors
- Cultural/behavioural factors
In addition to these four major divisions of cause, others have proposed subdivisions of some of these, combinations and new theories. MacIntyre also notes that there are literal and more liberal interpretations of the Black Report’s four causal paths and these are briefly outlined [100].

2.4.1 Artefact

The literal interpretation of the artefact explanation is that there is no real relationship between health and SES, it is simply an artefact of the measurement of health and of SES. The more liberal interpretation is that the magnitude of the association may be an artefact of the measurement of both health and class and may relate to the relative shrinkage of the poorest occupational classes. However, Fox et al found little support for the artefact explanation [101].

2.4.2 Natural/Social selection

The literal version of this explanation suggests that health determines class and less healthy people drift to the lower social classes, which thus have the highest mortality. The more liberal version suggests social class affects health, but that selection may explain some of the observed gradient. The Black Report concluded that there was little evidence for drift and its contribution to health inequalities was likely to be small [62]. Selection on the basis of health may be one cause of social mobility, which is itself separated into intergenerational social mobility (change of SES between parents and children) and intragenerational social mobility (change of socio-economic position after entry into the labour market). Social mobility may in turn be mediated not just by health outcomes but health behaviours (smoking, nutrition), structural/environmental factors (occupational exposures) and psychosocial stress-
related factors (life events, lack of social support) [64]. While Dohrenwend concluded that in general social causation was more important than social selection in generating a socioeconomic health gradient, schizophrenia in young adults was an exception, with selection being relatively more important [102].

**2.4.3 Cultural/Behavioural explanation**

The literal version of this explanation is that the association between SES and health is completely explained by health damaging behaviours, such as smoking, poor diet, lack of physical activity and inappropriate use of health services. The more liberal version suggests that these behaviours contribute to the social class gradient in health, but provides no explanation for why there should be a social class variation in these behaviours. Notably, the Whitehall studies found a residual SES effect on health, after adjustment for health damaging behaviours [74, 78]. Lantz suggests that the major health-damaging behaviours explain only a modest portion (10-20%) of the SES health gradient [103]. More than twenty years ago Berkman and Syme suggested these negative health behaviours may be a form of coping with stressors hypothesized to be associated with lower SES [37].

**2.4.4 Materialist/structural explanation**

This explanation emphasises the role of economic and associated structural factors in the distribution of health and well-being. Thus poverty or economic deprivation directly influences health through lack of access to health care or health information as well as higher exposure to disease-causing processes. The Black Report includes the notion of relative poverty in those who have sufficient for their most basic needs but lack the means for participation in a wealthy society.
Access to health services

The Black Report noted that the richer occupational classes made more use of the National Health Service than did poorer occupational groups, particularly preventive services. Poorer occupational groups consulted their primary health care providers less than their counterparts in richer groups when indicators of their greater needs were taken into consideration [62]. Thirty years ago, Tudor Hart described this “inverse care law” and has recently noted that it “is not a law of nature but of dehumanized market economics. It could be unmade by a rehumanized society” [104].

Research in Australia indicates that men and women in low-income families report more hospital episodes, outpatient visits and doctor visits than higher income families. However there is less use of preventive services [105]. Superficially, countries with national health systems provide adequate access to health care for all. In reality, increasing evidence indicates differences in the quality and appropriateness of medical care, and differential uptake and access to preventive services, may account for some of the socioeconomic health gradient [32].

Medical care has been likened to “the ambulance waiting at the bottom of the cliff” [106]. It does not determine who falls, though it may alter the outcomes for those who do [3]. As Sparks notes, “the fact that we get an equal chance of being cured once we’re ill is no compensation for the fact that our chances of becoming ill in the first place are unequal anyway” [107].
Research evidence suggests that while access to health care may be decisive in individual cases, the availability of such services – or their lack – cannot begin to explain the observed SES gradients in the health of populations [108], [109, 110]. Furthermore, despite universal access to medical care, there is differential uptake of the benefits of medical services and health education [111], [109], [106], so that the determinants of access become important.

While the Black Report makes no specific mention of a psychosocial theory of causation, MacIntyre suggests that the liberal version of the materialistic/structural explanation is that it is not only physical conditions that vary with SES and determine health, but psychosocial factors and social as well as economic capital. Implied psychosocial influences are indeed scattered throughout this section of the Report, e.g. social circumstances may determine access to dietary information; levels of job satisfaction and security as well as mental strain are noted as aspects of occupational class that may affect health; and the notion that unemployment may increase mortality through the “ill-defined concept of ‘stress’” is noted. I have included the psychosocial hypothesis under the materialistic/structural explanation, recognizing that some researchers would regard these as competing, rather than nested explanations.

**Psychosocial factors**

By the time the Black Report was published, the theory that both physical and psychological stress could increase vulnerability to disease, was already well-developed [36]. Cassel postulated that the social environment, including dominance
hierarchies, social disorganization, social isolation and rapid social change, could alter host susceptibility to disease via the neuroendocrine system. Positive social support and various psychological assets could buffer such effects.

The hypothesis that psychosocial factors may cause the socioeconomic health gradient proposes that although the basic material requirements of health (such as food and clean water) may be met, lower SES people suffer higher levels of stressor exposure through financial strain, monotonous work, greater number of stressful life events etc [112], [113]. In addition, individuals of lower social status may suffer greater distress for a given level of stressor exposure, as a result of poorer coping skills, lower self esteem or feelings of mastery that may be differentially distributed by SES [114], [113]. Greater levels of social support may be able to moderate both the level of stressor exposure and the health effects of greater stress experienced by lower SES individuals (Pearlin and Aneshensel, 1989 cited in [34]), but lower SES may be associated with lower levels of social support [113]. Thus the physiological impact of a stressor is subjective, depending on the objective features of the stressor, as well as the availability of buffering resources [113].

An interesting, indeed important, characteristic of the socioeconomic health gradient is that it has been persistent over several centuries, even though the causes of death have changed radically. Even over the last one hundred years this is so – at the beginning of the 20th century infectious diseases were important causes of mortality and age-standardised mortality rates were higher in lower socioeconomic groups. By the end of the century, heart disease and cancer are major causes of mortality, and
apart from a few exceptions they are also more common in lower socioeconomic groups [3]. Evans writes, “While death is ultimately quite democratic, deferral appears to be a privilege correlated with rank. The diseases change, the gradient persists.” Being in a lower SES group appears to be associated with some kind of increased susceptibility for disease, perhaps in addition to increased exposure to risk factors for specific diseases.

The finding from the Whitehall studies that measures of job stress could account for a large part of the socioeconomic gradient in the incidence of coronary heart disease suggested that not only could psychosocial factors alter susceptibility to other risk factors, they may be directly pathogenic [78], [75], [115]. Bosma also found that participants who had changed from a work environment of low job control to one of higher job control had lower risk of coronary heart disease than those who had remained in low control work. This suggests that policy responses targeting psychosocial factors such as job control may be successful, at least for cardiovascular disease, in improving health [75].

Psychosocial factors may have direct and indirect effects on health. Direct effects are caused by increased psychosocial stress and moderated by psychological assets and social support (covered in detail in Chapter 3). Indirectly, psychosocial factors may act through health damaging behaviours and decreased “access” to medical services – through decreased recognition of the need for health care, failure to use preventive services and lack of uptake of health promotion [116], [117].
Both ecological and individual studies provide support for the importance of psychosocial factors to health. In particular, the relative income hypothesis has as its basis a psychosocial causation of disease (see section 1.3.3) – above some absolute level of income, it is less how much you have, but how much you have compared to others that is important for health. While there is considerable debate around the relative income hypothesis, there is also considerable support for the importance of psychosocial factors in explaining the socioeconomic health gradient, from individual studies.

Bosma found that self-rated health in adulthood was associated with childhood SES, i.e. lower childhood SES was related to poor self-rated adult health, independent of adult social class (OR 1.67, 1.02-2.75) in 2174 men and women aged 25-74 years. Lower childhood SES was related to personality variables, with external locus of control (42% vs 15%), parochialism (35% v 12%) neuroticism (34% vs 23%) and lack of future orientation (41% vs 27%) more prevalent in the lowest compared with the highest social classes in childhood. External locus of control, neuroticism and the absence of active problem focused coping explained about half of the association between self-reported health and childhood SES [118].

All-cause mortality in Lithuanian men is much greater than that amongst men in Rotterdam. In the Kaunas-Rotterdam study, Bosma showed that Lithuanian men were substantially less optimistic with respect to their health and this factor (statistically) accounted for 30% of the excess in all cause mortality in Kaunas (cited in [119]). In a longitudinal study, Power et al found that two of the most important factors
explaining the poorer self-rated health of men and women in lower social classes compared to those in higher social classes were adolescent socioemotional adjustment and current level of job strain [120].

The psychosocial hypothesis of causation requires differential distribution of psychosocial factors by SES. Ruberman demonstrated that social isolation and a high degree of life stress were inversely related to SES (as assessed by education) and were positively associated with increased mortality following a myocardial infarct [121]. In the 1958 British birth cohort, there was a significant social class gradient in psychological distress in women by the age of 23 [122]. Job insecurity, higher job strain and low social support were more common in women in lower SES groups. In one hundred healthy middle-aged men and women, those in lower occupational prestige positions experienced more interpersonal conflict but there was no association between occupational prestige and measures of work strain [123]. The Whitehall studies found a socioeconomic patterning of job control, social support and social networks, with less favourable patterns in lower SES groups [124].

Studies of non-human primates provide support for the importance of psychosocial factors to health and their differential distribution by social hierarchy. Sustained social subordinance in stable dominance hierarchies in captive primates was associated with depression of the HDL:LDL ratio and increased stenosis of the coronary artery. Higher-ranking olive baboons had more efficient cortisol and sympathetic nervous system (SNS) responses to stress, while lower ranking individuals had a higher basal level of cortisol, consequent upon frequent activation
of the stress response [125]. A recent meta-analysis of studies examining primate dominance hierarchies and stress physiology demonstrated that the association of cortisol levels with rank varied for different primate species, depending on the social environment experienced. Subordinates in aggressive societies suffering high rates of physical and psychological stress exhibited much higher basal cortisol levels than dominants, whereas in non-aggressive societies subordinates had lower basal cortisol levels than dominant individuals [126]. Despite interspecies variation, consistent predictors of high cortisol levels in subordinate animals (compared to dominants) were higher rates of stressor exposure, decreased social support and lower interaction with close kin.

The psychosocial hypothesis suggests an increased susceptibility to disease that would explain the persistence of the health gradient, despite changes in the particular diseases experienced over time. It provides an explanation of why those who are not impoverished, not apparently lacking access to health care or the basic necessities of life, but occupy a lower social group, might have poorer health than those occupying higher social groups. Considerable epidemiological evidence exists of the importance of psychosocial factors in human disease — social isolation rivals smoking as a health risk [32]; acute stress may precipitate myocardial infarction and sudden cardiac death [127]; chronic stress (particularly occupational stress) is associated with an increased incidence of coronary heart disease [75] and psychological dispositions such as hostility and pessimism are predictive of all cause mortality [128],[129].
Although there is biological plausibility for the association of health with psychosocial factors, biological plausibility is only one of Hill’s criteria for causality [130]. Furthermore, most of the studies linking psychosocial factors to health are cross-sectional and self-reported chronic stress is liable to confounding by poor health [32] (although even studies using objective measures of job strain found associated health effects) [75, 131, 132]. Longitudinal studies are needed to confirm that chronic stress, lack of social support and adverse personality styles do indeed precede physiological changes that lead to disease.

Since the Black Report, a number of other hypotheses have been generated to explain the socioeconomic health gradient. The “programming” hypothesis and the life-course explanations focus on the timing of the insults that result in disparities in health in adulthood. The “programming” hypothesis ascribes health disparities purely to biological effects on intrauterine and postnatal development. The life-course theories incorporate both biological and psychosocial effects over the whole of the lifetime.

2.4.5 The “programming” hypothesis

Geographical variations in mortality in the United Kingdom have been recorded since the first national statistics were collected. Farr ascribed these geographical patterns to variations in provision of sanitation and clean water [33]. In the 1980s, Barker et al investigated regional differences in mortality from ischaemic heart disease in England and Wales and found that such variations appeared to be related not to current conditions, but to differences in infant mortality seventy years previously [133].
Using detailed birth records from as far back as 1911, researchers were able to trace 74% of males born between 1911 and 1930. Subsequent analysis revealed that men weighing less than 5.5 pounds at birth had the highest death rates for ischaemic heart disease and chronic obstructive airways disease. The weight at one year was also significant, with standardised mortality ratios (SMRs) of 111 in men who weighed ≤8.2 kg at one year, compared to 42 in those who weighed ≥12.3 kg at one year [134].

Studies in a number of countries have confirmed the importance of the intrauterine and early childhood environment [135], with different diseases having associations with differing periods of life. Thus cerebrovascular disease appears more closely related to intrauterine life [136], while chronic obstructive lung disease may be more related to the environment in the first year of life [137]. Cardiovascular disease is associated with both intrauterine and early postnatal life, possibly through effects on systolic blood pressure [137]. Lower birth weight and lower weight at one year are associated with increased risk of impaired glucose tolerance in adult life [133].

Birth weight itself appears to be of some importance. Key determinants of birth weight are the mother’s weight before she becomes pregnant and her own birth weight [138]. This means that the effects of low birth weight could be perpetuating and it may take several generations of improved circumstances to overcome the handicaps associated with intrauterine malnutrition. Such perpetuating risks have been noted in studies of women suffering famine in the first or second trimester of
pregnancy during the Dutch Hunger Winter of 1944-45. Not only were the offspring of these women of low birth weight and subsequently suffered a relatively high incidence of diabetes [135], but they in turn gave birth to babies of lower birth weight than those whose mothers had not been exposed to famine [139].

The proponents of the importance of intrauterine and early neonatal development to health in later life argue that health is programmed at this early stage, and there are critical periods in development during which adverse circumstances can have lasting effects on health [133]. The risk of hypertension, diabetes and heart disease in adulthood is linked to the combination of thinness at birth, and rapid postnatal growth [135]. Fetal growth, and growth and nutrition in infancy may have lasting influences on plasma concentrations of cholesterol, apolipoprotein B and fibrinogen, blood pressure, body fat distribution and liability to impaired glucose tolerance and diabetes [136].

There are plausible biological mechanisms by which the intrauterine environment could set the scene for later health, with some support from animal experiments [135]:

- Maternal malnutrition may trigger an increase in stress hormones in the mother, with disruption of placental growth, breakdown of the placental barrier and transfer of maternal stress hormones to the fetal circulation. This in turn may affect organ development in the fetus with later development of hypertension and vascular disease.
- Fetal malnutrition consequent upon maternal malnutrition may result in diversion of nutrients from other organs to the growing brain, resulting in abnormal
development of the liver, kidney and pancreas and increased risk of disorder in later life. In addition, fetal malnutrition may result in a decrease in the number of telomeres present in DNA. Telomeres, at the end of chromosomes, dictate the number of possible cell divisions, so that a decreased number implies cells may stop dividing prematurely, with subsequent loss of function in affected organs.

Criticisms of the programming hypothesis focus on the possible confounding effect of early-life social class and continuing social disadvantage in research studies [136]. Low birth weight is associated not only with higher morbidity and earlier death but with lower social class [138]. Low birth weight may simply be a marker of lower social class and the higher mortality in adulthood of low birth weight babies may be a reflection of social class, rather than birth weight per se. A recent report indicated that babies born to parents from social class I (highest SES) weigh on average 130 g more than those born to class V and 200g more than babies registered by their mothers alone [138]. In addition, there is a steep social class gradient associated with breast-feeding [138] and early infant feeding may have effects on lipid metabolism throughout life [133, 134].

Some research suggests that the associations between early weight and later risk factors are independent of SES, either currently or at birth [136]. The lack of an association between birth weight and social class at death argues against the persistence of an adverse environment from intrauterine life to death [134]. In addition, the specificity of different diseases for effects operating at different stages of infancy argues against this being simply an effect of continuing adversity.
It is clear that there are long-term sequelae of intrauterine malnutrition [140]. It also seems likely that these long-term consequences are independent of social class. What is less clear is whether it is possible to modify the risk of long-term consequences of intrauterine malnutrition, by influencing the postnatal environment.

The gradient in health associated with SES is finely graded – this is not a difference in health between the poor and the rich, but a difference in health along the whole socioeconomic range. While Barker’s work indicates a greater health risk for those under 5.5 pounds at birth the average birth weight was 7.9 pounds. Can this explanation of the origins of adult disease create the fine-grained differences that are observed, or are there other influences over the intervening time between babyhood and adulthood?

### 2.4.6 Life-course theories

Life-course theories emphasise the importance of influences operating throughout life. Two alternative models suggest different routes by which life circumstances may affect health, either by determining the range of possible subsequent life trajectories (pathway models) or by an accumulation of adverse effects (accumulation models).

**Pathways models**

In contrast to the programming, critical periods or latency hypothesis, the pathways model of health focuses on not only the early environment, but the moderating effects of subsequent life trajectories. Thus early life advantage or disadvantage sets the
trajectory into and through adult life [141]. Life is a series of pathways choices and each prior choice determines possible later choices [142].

Increasing research evidence suggests that health outcomes in adulthood are the end result of ‘chains of risk’ or ‘unhealthy life careers’ [143]. Of particular interest are studies that examine “resilient” individuals: people who experienced economic disadvantage in early life, but do not develop patterns of ill health in adulthood. Singer et al found a trend to increasingly adverse physiological profile with increasing economic disadvantage both currently and in childhood. But more importantly, some defied this trend - people with strong positive relationships with parents and partners. These resilient individuals did not follow a path of disadvantage, but started on a path of economic disadvantage, which could then be altered by other intervening advantages [144]. The researchers stress the importance of considering not only cumulative disadvantage, but also cumulative advantage, from starting resources (e.g. growing up in an intact family), biological endowment and positive movement through life transitions. Pathways to health outcomes are postulated to depend on the interplay between adversity and advantage, and this is what gives the fine grain of health inequalities.

**Accumulation models**

In accumulation models, exposure to disadvantage at different life stages has a cumulative effect on health [143]. Early disadvantage can be modified by later life experiences and the accumulation of adverse circumstances has a dose-response effect on health [145]. From origins in lower social classes, individuals may begin
with biological disadvantages such as low birth weight; perhaps have an increased risk of childhood psychological adversity and exposure to adverse environmental influences (e.g. tobacco smoke). There are likely to be fewer educational investments, poorer working environments, lower expectations and perhaps acceptance and encouragement of adverse health behaviours such as smoking, lack of exercise and poor diet.

Using the 1958 British birth cohort, researchers showed that birth weight was related to subsequent social circumstances from childhood to adulthood, suggesting that the effects of birth weight on subsequent well-being cannot be isolated from the childhood and adulthood environment; rather they tend to be intimately entwined [146]. Low birth weight should instead be seen as a marker of a disadvantaged life trajectory with later health being affected by the accumulation of risk, both social and biological [147]. A Finnish study incorporating measures of childhood and adult socioeconomic status found that poor adult health was associated with the combination of low childhood and low adult SES, but that those men who went from low childhood SES to high adult SES had the same mortality risks as those who were in high SES groups at both time points [148]. Similarly, Davey Smith reported strong positive trends to elevated blood pressure, angina, bronchitis and body mass index in men who were in manual occupational classes at three life points, compared to men who were in non-manual classes at all time points [149]. This effect appeared to be more apparent for cardiovascular disease than for cancer. Socioeconomic position in early and later life contributed independently to disease risk, but the association with cumulative social class was independent of the order in which the social class
position came, suggesting that social mobility or selection was not contributing in a significant manner. The postulated dose-response relationship in physical, psychological and cognitive functioning in association with the number of periods of economic hardship experienced during 1964-1983 has been demonstrated [145].

The timing of adverse influence such as low SES may be important, with Blane et al finding that childhood SES was associated with physiological risk factors, e.g. BMI, serum cholesterol, blood pressure and FEV1, while adult SES was more closely associated with behavioural risk factors (exercise and smoking) [150].

The life-course approach includes aspects from each of the individual-based theories presented above. Childhood disadvantage may be genetic, congenital (due to intrauterine influences), psychosocial and material. Exposure to adverse early childhood conditions may then influence educational opportunities, psychosocial development [113] and subsequent employment. But the effects in childhood are not irreversible as the programming hypothesis suggests, and there is evidence that initial disadvantage can be buffered by a supportive early environment [3]. Even genetic predispositions may depend on the environment for their expression and this is apparently true also of intrauterine insults. In the Kauai Longitudinal study, children who had suffered moderate or severe perinatal stress, but were subsequently reared in “good” environments (stable family of high socioeconomic status) suffered little or no disadvantage in development at twenty months (cited in [3]). Children with no perinatal stress likewise showed little disadvantage despite poor, unstable households.
But two negative factors together had quite severe consequences for child development.

The hypotheses presented above are largely focused on the individual – individual health behaviours, levels of stress and social support, exposures in utero and over the life-course. But individuals are members of a society and subject to the social influences of which they are a part. Exposure to physical and psychosocial risk factors is shaped by the social, political and economic environment. To focus only on the individual in theorizing the causes of socioeconomic health inequalities is “a bit like studying why some people swim well and others drown when tossed into a river and this displaces study of who is tossing whom into the current” [151].

While the current study is based on individuals, each individual reflects the experiences of the hierarchy of social, economic and political influences. These more upstream theories are briefly outlined below.

### 2.4.7 Neomaterial explanation

Lynch et al favour an explanation for socioeconomic health inequalities in which, “health inequalities result from the differential accumulation of exposures and experiences that have their sources in the material world” [152]. Policies that favour dismantling of the welfare state and systematic under-investment in human, physical, health and social infrastructure expose individuals to physical, psychological and social stressors.
Proponents of the neomaterial explanation have been critical of Wilkinson’s relative income hypothesis and the importance of income inequality to the health of whole populations. They have found little support for the importance of population level psychosocial attributes, e.g. social capital and sense of control over life, to differences in health between countries [50]. Rather they emphasise the importance of the economic environment and the historical and cultural context that has generated it.

At an individual level, neomaterialists suggest that the economic environment works through physical, behavioural and psychosocial pathways to affect health. The neomaterial explanation represents an attempt to look at the more upstream causes of health inequalities, but one final pathway of effect is similar to that postulated under the psychosocial hypothesis outlined above. This allows explanation for multiple disease outcomes through multiple mechanisms and the maintenance of a socioeconomic health gradient, even when the intervening, disease-producing mechanisms, change [9].

2.4.8 Social production of disease and/or “the political economy of health”

These theories focus on the social and political production of disease with an emphasis on the social, political and economic environment, rather than individual characteristics and behaviours. Socioeconomic status is a fundamental, or root cause of inequalities in health – and SES itself is seated in the economic and political institutions and decisions that create and enforce it [151]. Hand in hand with the creation of widening SES disparities is the dismantling of the welfare state and safety
nets that shield the disadvantaged from the effects of major life events such as employment or ill health.

Examples supporting the central nature of social, political and economic circumstances include observations of stalled health improvement in countries which have had large increases in GNP (but no change in social policies), and in countries and states that are relatively poor, but have high levels of social investment and little SES health gradient (e.g. Kerala in India and Costa Rica) [51], [73].

Social capital and social cohesion are hypothesized to be eroded by political and economic processes and affect health through psychosocial effects such as increased negative emotions of shame and distrust, directly via the neuroendocrine system or indirectly through health damaging behaviours, such as smoking [51]. These theories suggest that moves to increase social capital without altering the underlying determinants may be ineffective and the equivalent of a community level version of “blame the victim” [51].

2.4.9 Ecosocial theory and related multi-level dynamic perspectives

Multi-level theories, such as the ecosocial theory, eco-epidemiology and social-ecologic systems perspective embrace the economic, social and political determinants of health as macro-level factors, as well as individual determinants of health as micro-level factors. As their names suggest, these theories propose health is the result of multiple levels of influence over the life-course, finally being embodied in the
individual through biological pathways that have been shaped by evolutionary history [151].

Influences on individual biology come from all levels (individual, neighbourhood, regional or political jurisdiction, national or international) and in multiple domains (home, work, school and other public settings). Although public health is concerned primarily with the health of populations, population health is itself an aggregate of the health of individuals. One facet of our understanding of population health must come through the investigation of influences on individual biology.

2.5 Biological Pathways

The final pathway for all theories that invoke (at least partly) a psychosocial hypothesis of causation is through the embodiment of physical, social and psychological factors via biological pathways. This is outlined in Figure 2.2. It is this final step that the thesis seeks to investigate more fully.

The neuroendocrine response to stress is well characterized and it is clear that this is a response to physical as well as perceived psychological stress. (An overview of the stress response is presented in Chapter 3, with a more detailed coverage in Appendix A). However, there is little research on the socioeconomic distribution of psychological and social assets that are thought to modulate the stress response as well as the socioeconomic distribution of stress itself.
It may seem logical to assume that those in lower SES groups are more stressed, but it may be less obvious that this is a gradient of stress and psychosocial assets, which could account for the SES gradient in health.

Although there are known links between the central nervous system (including elements of the neuroendocrine stress response) and the immune, haemostatic and metabolic systems, the effects of stress on these systems is still being elucidated.
Laboratory stress is well studied, but in general researchers examine acute effects on the immune and metabolic systems in association with acute stress. In contrast, socioeconomic disparities in stress (as postulated by the psychosocial hypothesis) are likely to be evident particularly for chronic stress e.g. perceptions of financial hardship, lack of control in the workplace.

In a review of the “biology of inequality”, Brunner notes the biological outcomes of early life stress and their importance for physiological change over the life-course. Using results of animal and physiological experiments he makes a plausible case for the role of continuing psychosocial adversity and personality assets in the causation of the socioeconomic variation in health [6].

In 1997, Kelly et al called for “population-based, person-specific health surveys, with concomitant biological measures” providing information on socioeconomic and psychosocial factors to investigate whether these are in fact embedded in physiology in a way that could be associated with future disease. The paper detailed the requirements for such biological measures and is reviewed here in detail [154].

Two primary conditions are required of such biological measures:

1. they should be sensitive to systematic, long term differences in socioeconomic status and living conditions

2. they must be feasible to measure in large scale population surveys

To these requirements, I add two further primary requirements:
1. they should be plausibly associated with psychosocial factors
2. they should be plausible antecedents of disease

To confirm the embodiment of psychosocial factors that vary by SES, biological measures should be proven to be altered by these psychosocial factors. Secondly, if the measures that are chosen are logical forerunners to disease, then the pathway from SES, via psychosocial factors, to disease will be clearer.

Steptoe has outlined a schema for the embodiment of stress, giving examples of the types of physiological variables that may be altered, and thus could be measured for this purpose. This is presented in Figure 2.3.

Kelly et al further describe the criteria required for markers of the chronic stress response:

The criteria proposed for the ideal marker are:

1. It must be affected/ caused/ created by stress;
2. It should have a long half-life (months to years). It should not require repeat measurements;
3. It should be independent of demographic factors (e.g. sex or age) or else have a well-defined demographic distribution;
4. It must be relatively easy and cheap to measure (preferably with an easily reproducible, automated method);
Figure 2.3. Schema for the biological pathways by which stress is embodied. (adapted from Steptoe (1998) The psychobiological basis of disease.[155]
5. It should require no special sample preparation; and
6. It should have readily available reference standards.

Kelly’s review went on to consider the qualities of possible markers. Using this review as a basis, and the criteria outlined above, I chose five biological markers to examine the possible biological pathways from socioeconomic status, via psychosocial factors, to ill-health.

2.6 Conclusion

Recent research provides considerable support for the role of psychosocial factors in the causation of health inequalities. The evidence is strongest for socioeconomic health inequalities, but psychosocial factors may also play a role in gender and ethnic health inequalities.

Several of the competing theories of causation are only superficially competing – disparities in access may work partly through psychosocial avenues; health damaging behaviours may have their origin in psychosocial influences; effects of the social, economic and political environments, at their individual levels work through psychosocial channels as the final common pathway to ill health.

For psychosocial factors to be on the causal route from socioeconomic status to health, they themselves (or the body response to them) must vary by SES and there should be physiological evidence of their effects on body physiology in multiple systems. Plausible biological markers of systems postulated to be affected by
psychosocial factors should thus also show a socioeconomic gradient. Evidence of a link between these biological markers and subsequent disease would provide support for pathways between SES, psychosocial factors and disease.

This thesis thus tests the following three hypotheses:

1. Measured psychosocial factors vary by SES
2. For each of five biological markers there is a gradient associated with SES
3. SES variation in the biological markers is mediated by psychosocial factors.

In the following chapter I begin by reviewing the physiological stress response and the intercommunication between elements of the stress response and other body systems. I introduce the markers and describe how they fit into the stress response and their plausible association with subsequent disease susceptibility.
Chapter 3.

Part One: Background physiology

3.1 Introduction

A psychosocial explanation for the socioeconomic gradient in health invokes the notion of a differential distribution of stress across socioeconomic groups. In order to select appropriate biological markers to investigate the psychosocial explanation, we need to understand something of the physiology of the stress response. The following chapter reviews the current literature on the neuroendocrine response to stress and the physiological consequences of this response for body systems. A more detailed description of the stress response and its effect on body systems is contained in Appendix A.

3.2 Stress – a definition

As originally used in a biomedical sense by Selye and Wolff, the term stress referred to “that state within a living creature which results from the interaction of the organism with noxious stimuli or circumstances, i.e. it is a dynamic state within the organism” (cited in Cassel, 1976 [36]). Combining this with Cannon’s postulate of homeostasis [156], in this thesis, stress is “a threat, real or implied, to homeostasis” [157]. Something that poses a threat is a stressor; the body’s response is the stress response. Stressors may be physical or emotional and include both stressors causing “distress” (stress in the negative sense) as well as the exhilaration of “good” stress. Activation of the stress response leads to behavioural and physiological changes that
improve the ability to maintain homeostasis and provide defense against perceived dangers.

3.3 Stress, stressors and the stress response

The cycle of the physiological response to a stressor contains several elements, illustrated in Figure 3.1.

Stressor exposure: The stress response may be activated by any type of stressor exposure, whether physical, e.g. a marathon, psychological, e.g. academic examination, or biological, e.g. injury with blood loss. Each type of stressor precipitates a similar response, referred to by Selye as “the general adaptation syndrome” (cited in [158]).

Perception of threat: Some stressors will be universally perceived as threatening, such as physical attack. However, other stressors will be experienced as threatening to some, but not to others, e.g. public speaking. The perception of threat by psychological stressors is particularly varied: what is stressful to one individual is exciting and empowering for another. Social support, coping skills, self esteem and previous life experiences probably all play a part in altering the perception of a threat.

Neuroendocrine response: The general neuroendocrine response to stressor exposure is modulated by inputs from adjacent brain areas encoding memory, reason and personality, ensuring a response that is specific to an individual.
The extent of the stress response is proportional to the intensity of the stimulus [158] and is usually somewhat specific to the stressor, e.g. the coagulation cascade is not activated during psychological stress. However, the response can become non-specific if the stressor exceeds a threshold in magnitude or duration [159].

Maintenance of homeostasis: Cannon first postulated the concept of homeostasis in 1929, to describe the recurrent minute physiological adjustments required to maintain a constant internal milieu, essential for metabolic processes [156]. He further suggested that the body responds to a threat to homeostasis by activating a series of interconnected neurohumoral pathways, which he termed the fight or flight response. Fight/flight has been the classical stress response, understandable in evolutionary...
terms as a response to a physical threat. We now know that fight/flight represents only one part of the stress response, the immediate reaction to stressor exposure that is mediated by the sympathetic nervous system and the adrenal medulla (the sympathetic adrenal medullary system, SAM). This response mobilizes the immune system, the haemostatic system, the metabolic system and the nervous system to react optimally to restore homeostasis.

More recently researchers have identified a slower stress response, occurring over minutes to hours, which is intimately connected to the immediate stress response, but is activated particularly in circumstances where a stressor is perceived as overwhelming [5]. A number of authors describe a “defeat response” resulting in activation of the hypothalamic pituitary axis (HPA), with a secondary role for the sympathetic nervous system [160, 5]. In most situations both the HPA and the SAM system are activated and are equally important to survival – indeed they are intimately interconnected and activation of one triggers activation of the other.

Recently, Taylor et al hypothesised that a more effective response to a physical threat throughout evolution, particularly for females, would have been a “tend and befriend” response, rather than a fight or flight response. Females who were pregnant or caring for offspring might be less able to fight or flee. It would be more practical to remain calm, soothe offspring and develop strong social ties ensuring a supportive and protective group. There is some physiological evidence to support this hypothesis, via activation of oxytocin secretion [161]. However, this research is in its infancy, and although consideration of this type of response may explain some gender differences
in responses to stressor exposure, the remainder of this chapter concentrates on the fight/flight response and the response of the HPA.

Turning off the stress response: An efficient stress response is appropriately triggered, reaches an amplitude commensurate with the intensity of the threat and is turned off shortly after exposure to the stressor ceases. The entire response is tightly controlled by positive and negative feedback loops. Hence, the widespread effects of the stress response on the body are ordinarily transient and tolerable [158].

3.3.1 The neuroendocrine stress response

The neuroendocrine response to stress is complex and the understanding of its components and their interactions is continually being refined. An overview of the neuroendocrine stress response is presented below, with a brief outline of its widespread effect on multiple body systems, in order to position the chosen biological markers within the stress response framework. A more detailed account of the response (including a description of the functioning of the body systems involved) is presented in Appendix A.

The early stress response occurs via activation of various elements of the central nervous system. The autonomic nervous system (ANS) receives inputs from higher centres involved with memory, emotion, anticipatory phenomena, and cognitive functions and in turn exerts its effects peripherally via circulating catecholamines.
Box 3.1
Effects of activation of the sympathetic adrenal medullary (SAM) system during acute stress

2) Direct effects
   a. Cardiovascular – vasoconstriction with diminished blood flow to the gut, kidney and mucous membranes, but with maintenance of blood flow to the heart and improved blood flow to skeletal muscles; increased heart rate and cardiac output
   b. Metabolism – increased metabolic rate; accelerated fuel mobilization by lipolysis and gluconeogenesis.
   c. Fluids and electrolytes – maintenance of extracellular fluid volume by action on the renal tubules; promotion of cellular uptake of potassium.
   d. Viscera – bronchodilation; stimulation of bladder and gut sphincters.
   e. Immune – induces secretion of IL-6

3) Indirect
   a. Endocrine system – influence the secretion of renin, erythropoietin, thyroxine and a number of other hormones, thus assisting in the homeostatic and allostatic feedback loops for these hormones.
   b. Insulin – suppression of insulin secretion and stimulation of glucagon release, supporting mobilization and availability of blood glucose.

4) Corticotrophin releasing hormone (CRH) is released from the paraventricular nucleus of the hypothalamus
Activation of the sympathetic branch of the ANS results in the release of adrenaline from the adrenal medulla; adrenaline in turn has multiple target organ effects, as outlined in Box 3.1. These effects cause the typical signs of the acute stress (fight/flight) response: accelerated heart rate, dilated pupils and improved blood flow to skeletal muscle. The central nucleus of the sympathetic nervous system also produces corticotrophin releasing hormone (CRH), providing one route of communication between the neural and endocrine arms of the stress response.

The primary site of activation for the slower endocrine stress response is the hypothalamus, which also receives inputs from brain centres for memory, emotion, cognitive function and anticipatory phenomena. CRH is released from nuclei in the hypothalamus and stimulates production of adrenocorticotrophin (ACTH) from the anterior pituitary gland. ACTH in turn stimulates the adrenal cortex to produce glucocorticoids, of which cortisol is the most important. Cortisol is released over minutes to hours and exerts its effects through widely distributed intracellular glucocorticoid receptors (GCRs). The effects of activation of the HPA are summarized in Box 3.2.
Box 3.2
Effects of activation of the HPA during acute stress

1) Direct effects
   a. Metabolism – increased blood glucose via gluconeogenesis; increased protein breakdown and lipolysis in muscles;
   b. Immune – elevation of circulating polymorphonuclear leukocytes, depletion of eosinophils and T cells; inhibit the production of IL-6;
   c. Reproduction – increased CRH (hypothalamus) and cortisol inhibits release of gonadotrophin releasing hormone and increases resistance of target tissues to sex steroids;
   d. Growth – suppression of growth hormone releasing hormone by CRH; cortisol inhibits the effects of insulin-like growth factor 1.

2) Indirect
   e. SAM – permissive effect on secretion of adrenaline;
   f. Insulin – cortisol has an antagonistic action in skeletal muscle, potentiates insulin effects on adipose tissue;
   g. Acute phase response – cortisol enhances the effect of IL-1 and IL-6 on acute phase protein production.

3) Cortisol inhibits secretion of ACTH and CRH.
The stress response is closely linked to the immune system: cortisol affects immune cell distribution and function; catecholamines activate release of cytokines (in particular IL-6); and the hypothalamus is stimulated by IL-6 to release further CRH. These connections are outlined in Figure 3.2.

**Figure 3.2** Communications between the brain, HPA and the immune system. (adapted from [29])
Interconnections between the central neural and endocrine arms of the stress response, and between different parts of the central endocrine arm, result in a positive, reverberatory, feedback loop so that activation of one part of the system activates the other [162]. This permits fine matching of the intensity of the stress response to the intensity of the perceived stressor. The response is damped down by negative feedback loops involving cortisol, causing inhibition of both the central nuclei of the SNS and the secretion of ACTH and CRH.

The immediate stress response is modulated by prior experiences and contributes to building memory and memory contexts for future reference, via reciprocal connections between the central nuclei of the SNS, the hypothalamus and higher centres. Such connections also promote enhanced arousal, alertness, vigilance, cognition, focused attention and appropriate aggression during the stress response itself [171]. Recent evidence shows that not only perceived stress, but also the memory or anticipation of stressful events incurs physiological changes such as increased cortisol levels [163].

### 3.3.2 Behavioural responses

In addition to the neuroendocrine response to stressor exposure, behavioural responses occur. These may be focused and appropriate, to diminish the threat posed by the stressor, e.g. flight. Other behavioural responses to stressors, such as eating, alcohol consumption, smoking and other substance abuse may occur when the threat is ill defined or imaginary and when there is no clear alternative behavioural response that would end the threat [164]. Such responses can exacerbate and potentiate the
production of the physiological mediators of health outcomes, e.g. smoking elevates blood pressure and accelerates atherogenesis, exacerbating the hypertensive effects of repeated activation of the stress response [165]. In addition, depression, anxiety, excessive alcohol consumption and smoking may directly sensitize the HPA axis resulting in an abnormal stress response [166].

### 3.3.3 Individual difference

Marked individual variability in neuroendocrine reactions to various stressors depends on the individual perception and interpretation of a situation [167]. This in turn is influenced by past experience, memory, coping strategies and the context of memories contained in the mesocorticolimbic system, the amygdala and the hippocampus. Prolonged stressor exposure may overwhelm coping resources with resulting psychological distress and depression [167].

A number of studies have identified high responders (HRs) and low responders (LRs) based on measures of SNS activity, such as heart rate or cardiac pre-ejection fraction. While the two groups show no differences in baseline measures of, for example, serum cortisol, HRs may show exaggerated SAM or HPA axis responses to stressor exposure and fail to habituate to a stressor compared to LRs [168]. Genetic factors may underlie at least part of this difference, but influences from very early life may also shape the neuroendocrine response [169].

The young brain undergoes rapid growth and a high turnover of neuronal connections during the prenatal and early postnatal period. Exposure to hormones of the stress
system in early life may have lasting effects on the developing brain and influence
behaviour and physiological functioning throughout life [158]. This brain plasticity
lessens during childhood and adolescence and plateaus in young adulthood. Early
exposure to stress hormones may underlie the important influences of prenatal
conditions for later health, outlined by Barker [133].

Individuals also differ in their physiological ability to respond to stressors, which is
determined by genetic factors, as well as behavioural and lifestyle choices [165]. For
example, atherosclerotic blood vessels consequent on a rich diet, smoking and lack of
exercise would be likely to respond poorly to typical stress-induced changes in blood
flow to muscle, thereby restricting the efficacy of the stress response.

Box 3.3 summarizes the behavioural and physical adaptations seen in acute stress.

3.3.4 Animal studies

In animal societies, rank correlates well with the efficiency of the stress response.
During times of social stability, dominant male olive baboons have a faster, larger
stress response than subordinate males, with faster recovery. They are more resistant
to the suppressive effects of the stress response on testosterone. Subordinates have
centrally induced hypercortisolism and show resistance to cortisol feedback inhibition
[170]. However, under conditions of instability of the dominance hierarchy,
previously dominant males no longer show the more favourable stress response, i.e. it
appears to be related to rank rather than inherent individual characteristics.
**Box 3.3**  Summary of behavioural and physical adaptations during acute stress

**Behavioural Adaptation**

Adaptive redirection of behaviour
- Increased arousal and alertness
- Increased cognition, vigilance and focused attention
- Euphoria or dysphoria
- Heightened analgesia
- Increased temperature
- Suppression of appetite and feeding behaviour
- Suppression of reproductive behaviour
- Containment of the stress response

**Physical Adaptation**

Adaptive redirection of energy
- Oxygen and nutrients directed to the CNS and stressed body site
- Altered cardiovascular tone, increased blood pressure and heart rate
- Increased respiratory rate
- Increased gluconeogenesis and lipolysis
- Detoxification from toxic products
- Inhibition of growth and reproductive systems
- Inhibition of digestion-increased colonic motility
- Containment of the inflammatory/immune response
- Containment of the stress response

Adapted from [171]
Studies in rats indicate that early stress and neonatal handling may set the level of responsiveness of the HPA axis and the ANS. Those exposed prenatally to unpredictable laboratory stress showed overreaction of these systems, while animals exposed postnatally to handling (involving the mild stress of brief daily separation from the mother) showed an under reaction of these systems, in response to a stressor [172]. There is a slower rate of cognitive aging and a reduced loss of hippocampal function in rats experiencing this recurrent mild stress as neonates [173].

3.3.5 An evolutionary perspective

Humans have evolved from relative simplicity to complexity - in their social behaviours, cognition, emotions, diet and physical wants/needs. It seems however that human physiology has evolved more slowly and the stress response that was a driving force to human survival when dangers to life were largely physical is not particularly suited to stressors that are chronic, psychological and do not require a physical response.

The body is primed for defense against traditional dangers – fierce animals, aggressive humans, and other environmental dangers. Indeed, bodily systems are more adapted to recurrent, episodic, acute physical stress with brief activation of the ANS and the HPA to enable fight or flight, followed by activity to replenish supplies of energy – increasing appetite and food-seeking behaviour. But the same stress pathways may be persistently activated by chronic or repeated psychological stress, with adverse consequences to health and well-being.
3.4 Allostasis and allostatic load

Cannon proposed the notion of homeostasis as the maintenance of internal conditions via a series of metabolic feedback loops involving nervous and endocrine systems [156]. More recently, Sterling and Eyer have advanced the theory of allostasis: the ability to achieve stability through change [174]. McEwen has subsequently refined and distinguished the theories of homeostasis and allostasis so that homeostasis concerns critical variables such as body temperature, blood oxygen and blood pH that must be maintained within a narrow range, through a series of metabolic feedback loops, to ensure cellular survival. In contrast, allostasis concerns other physiological systems that respond over a broader range, e.g. changes in blood pressure, to internal and external perceived or anticipated demands as well as circadian rhythms, as an adaptation to the environment [172].

Like homeostasis, allostasis operates through a series of metabolic feedback loops, but body systems providing defense, adaptation and recovery can be rapidly mobilized, operating set points and the balance between systems changed within a wide range, and then turned down when they are no longer required. Large changes in pulse and blood pressure within the fight/flight paradigm are simple examples of allostasis. McEwen suggests that the core of the body’s response to a challenge is twofold: turning on an allostatic response that initiates a complex adaptive pathway, and then shutting off this response when the threat has passed [172]. He has further developed the concept of allostatic load, which is “the wear and tear on the body and brain resulting from chronic over activity or inactivity of physiological systems that are normally involved in adaptation to environmental challenge” [172]. Such
challenges may be psychosocial or physical and allostatic load may occur as a result of chronic psychosocial stress where repeated inefficiencies in the allostatic mechanism lead to damage to body systems.

There are four types of physiological responses that might result in allostatic load:

**Type 1:** Frequent stress causes repeated mobilization of the allostatic system with the level of allostatic load dependent on the frequency and intensity of “hits” (i.e. repeated excess secretion of various mediators). Example: Frequent repeated elevations of blood pressure in response to stressor exposure cause damage to the cardiovascular system.

![Blood pressure graph](image)

**Type 2:** Lack of habituation or adaptation to repetition from the same stressors. Example: many people in whom serum cortisol is raised with public speaking will adapt to this stress, so that continued experience with public speaking leads to a loss of this cortisol elevation. However, other people do not become habituated and repeated exposure leads to repeated elevations of serum cortisol.
Type 3: Failed shut down following chronic activity. Example: women with a history of depressive illness experience decreased bone mineral density due to chronic moderate elevation of serum cortisol and subsequent inhibition of bone formation [165].

Type 4: Inadequate production of mediators in response to a challenge. This may trigger compensatory increases in other systems. Example: the blunted HPA response to stress seen in chronic fatigue syndrome may reflect this type of allostatic load.
The concept of allostatic load has been investigated in humans in the MacArthur Studies of Successful Aging. Using a theoretically derived panel of markers of allostatic load, Seeman et al [175] demonstrated that higher levels of allostatic load were associated with poorer cognitive performance ($r = -0.13; p < 0.001$) and increased risk for decline in memory, as well as poorer physical performance ($r = -0.09$, $p = 0.01$) and increased incident cardiovascular disease ($\chi^2 = 5.2; p = 0.07$). In addition, in this longitudinal study, higher allostatic load at baseline predicted increased risks for decline in cognitive and physical functioning.

### 3.5 The acute stress response vs chronic response to stressors

The chronic stress response is the summation of many large and small acute stress responses, perhaps with no opportunity for a return to the basal resting state between successive activations of the involved systems. However, acute and chronic stress may have quite different effects on body systems, e.g. although the acute stress response activates the immune response, in situations of chronic activation of the stress response, immunosuppression occurs; in acute stress there is mobilisation of energy stores to provide substrate to muscles, but with chronic elevation of stress mediators there is deposition of fat, insulin resistance and hypertension [176].

In the brain, glucocorticoids and catecholamines released in the acute stress response act in concert to promote the formation of memories of events of potentially dangerous situations or strong emotional overtones, as input to future responses to similar events. Chronically, however, stress hormones and glucocorticoids in
particular, contribute to impairment of cognitive function and promote damage to brain structures such as the hippocampus [164, 173].

McEwen refers to this as the protection-damage paradox, whereby in acute stress adrenaline and cortisol mediate a protective response from the immune system, the metabolic system and the haemostatic system. Yet chronic activation of the same response is associated with allostatic load and impairment of these systems [176].

Feelings of anticipation and worry can also contribute to allostatic load. Anticipation primes the reflex that prevents us from blacking out when we get out of bed in the morning and is also part of worry, anxiety and the cognitive preparation to cope with a threat. Anticipatory anxiety can drive the secretion of mediators like corticotropin, cortisol and adrenaline and for this reason prolonged anxiety and anticipation are likely to result in allostatic load [172]. Behaviours such as excessive alcohol intake contribute to allostatic load, in this case by impairing hepatic function, thus altering the ability of the liver to respond to normal physiological cues.

3.6 Conclusion

The acute stress response represents a finely honed physiological reaction to an acute stressor, refined over thousands of years of natural selection – those with the most efficient response survived stressors while others perished. Over the last few hundred years (at least in developed countries), psychological stressors may have overtaken physical stressors in frequency. But the acute stress response reacts in a similar way to stressors, no matter what their nature. In addition, humans now face more chronic
stressors – poverty, malnutrition, racism, long work hours, a materialistic society where more is better and communication systems allow each individual to be aware of all the “mores” that are available.

The nature and wide-ranging effects of the stress response indicate the biological plausibility of the psychosocial explanation of socioeconomic health inequalities. It also guides the choice of markers to examine the effects of chronic stress, as postulated under the psychosocial explanation of socioeconomic health inequalities. The following sections introduce the markers selected: their physiology and known associations with health, SES and stress.
Part Two: Biological Markers

3.7 Introduction

In the laboratory, the stress response is measured by changes in the levels of those hormones that are intimately involved in the neuroendocrine system, i.e. adrenaline and cortisol. Adrenaline is very reactive to acute stress, rising in seconds to minutes (including during taking blood), with a short half-life in blood and is thus not suitable for examining chronic stress or for population based studies. Cortisol can be measured in blood, urine or saliva but shows large diurnal fluctuations that vary from individual to individual. If urine is collected it should be at least a 12-hour collection, thus creating problems for participant compliance. Cortisol levels are also very responsive to acute stress, so that they are of less use in the measurement of chronic stress, while the individuality of the diurnal variation poses problems for population-based collection.

Kelly et al’s review concluded that likely candidates for markers of the stress response would be glycated haemoglobin (as a marker of the neuroendocrine system), possibly serum immunoglobulin concentrations or functional lymphocyte assays (as markers of the immune system), fibrinogen (as a marker of the haemostatic system) and waist-hip ratio (as a marker of the metabolic system) [154].

I chose to measure three of the biological markers suggested in this review – glycated haemoglobin, fibrinogen and waist-hip ratio. Functional lymphocyte assays were not
financially or logistically viable. In a review of immune markers of the stress response, Vedhara et al emphasise the importance of measuring multiple immune parameters, reflecting the complexity of the immune system [177]. One immune effect that has received a modest amount of research is the effect of stressor exposure on the susceptibility to the common cold [178]. Saliva is the first line of defense against pathogens invading the oral mucosa, with the principal antibody being salivary IgA. Canberra has a centre for research on mucosal immunology with expertise in the collection and measurement of salivary antibodies. Immunoglobulins reflect the activity of one branch of the immune system, so that a marker of the other branch (cell-mediated immunity) was sought. Neopterin is a small, biologically stable molecule, reflecting the degree of activation of T cells. It is measurable with commercial assays and a single study has shown that acute stress elevated, but while sub-acute stress depressed, serum neopterin.

Because the neuroendocrine stress response has wide ranging effects on body systems (see Chapter 3, Part One), in this study, five biological markers were finally chosen (three from Kelly’s review, as well as neopterin and salivary IgA) as representatives of the haemostatic system, the metabolic system and the immune system. The remainder of this chapter reviews the current knowledge of each of these markers: their physiology, association with disease, population distribution and issues in their measurement that are important in their use as markers of the stress response.
3.8 Fibrinogen

3.8.1 Physiology

Fibrinogen was first recognized and named in the middle of the nineteenth century as contributing to the formation of a blood clot. Malpighi had earlier recognized that the central structural component of a clot was a white fibrous substance, later named fibrin. Its circulating precursor was named fibrinogen [179].

Fibrinogen is synthesised by hepatocytes under the influence of proinflammatory agents such as IL-6 in the acute phase response (see Appendix A), with feedback inhibition by extracellular sterols (mixtures of cholesterol and 25-hydroxycholesterol) [180]. IL-6 and catecholeamines upregulate the synthesis of fibrinogen while glucocorticoids enhance the effect of IL-6 [181-183]. Smoking increases fibrinogen synthesis, possibly via a prolonged acute phase reaction and IL-6, or via stimulation of catecholamine release [183]. Activation of the acute phase response can result in a two to ten fold increase in plasma fibrinogen [180].

Fibrinogen is a dimer, composed of two identical sets of three polypeptide chains termed Aα, Bβ and γ, joined by disulphide bonds [184]. There are three genes coding for fibrinogen (FGA, FGB and FGG), corresponding to the three polypeptide chains, situated in a cluster on the long arm of chromosome 4 [185]. Synthesis of the Bβ chain is the rate-limiting step and is responsive to IL-6. There is some evidence that individuals with particular fibrinogen genotypes may be more likely to have elevated levels of fibrinogen [185].
Conversion of fibrinogen to form fibrin is the final step of the coagulation cascade, leading to formation of a blood clot, or thrombus. Following the thrombin-mediated cleavage of, firstly, the A peptide and then the B peptide, fibrin is formed and forms the basis of the network structure of the clot which also includes platelets, red blood cells, monocytes and a variety of growth and chemotactic factors released by these cells.

Fibrinogen has a number of roles besides being the precursor for fibrin.

**Platelet aggregation**

While the adhesion of platelets to vascular subendothelium is the initial event in primary haemostasis, continued platelet aggregation to form the haemostatic plug is mediated by circulating fibrinogen which links adjacent platelets via glycoprotein receptors [186].

**Leukocyte trafficking**

Fibrinogen may play a part in leukocyte movement from the blood into extracellular spaces in the acute inflammatory process. Leukocyte adhesion to fibrinogen may be crucial for the localization of leukocytes to sites of inflammation and the activation of a specific leukocyte response [187].

**Tumour progression**

Recent research has revealed abundant fibrin within the stroma of a variety of tumours. This fibrin may protect the developing tumour from infiltration by
inflammatory cells that would attack the tumour. Fibrin deposition is seen in small cell carcinoma of the lung, renal carcinoma and malignant melanoma. Within some tumours, fibrinogen (rather than fibrin) is present in the stroma. Mesothelioma, breast cancer, colon cancer and lymphoma have abundant fibrinogen and recent studies suggest that it may promote the formation of new blood vessels (angiogenesis) allowing the tumour to grow and metastasise [188].

**Association with lipid metabolism**

Hepatic cells secreting more fibrinogen also secrete more apoB, a large protein that is the principal structural protein in low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) cholesterol [180]. Elevated plasma fibrinogen is associated with abnormalities termed “the metabolic syndrome”: raised fasting insulin concentrations, low high-density lipoprotein (HDL), high levels of low density lipoprotein (LDL) and high levels of triglycerides [189].

**3.8.2 Association with disease**

**Cardiovascular disease.**

Many studies have now shown that elevated levels of fibrinogen are independent risk predictors for cardiovascular disease [190-193]. Some studies indicate a steady rise in cardiovascular risk with increasing levels of plasma fibrinogen; an increase of 1g/L has been associated with a 45% increased risk of myocardial infarction [192]. Other research indicates that there is a threshold effect, with increased risk (relative risk = 2.09, 95% CI 1.15-3.78) only at the highest levels of fibrinogen (>90 percentile of the distribution among control subjects) [193]. In a recent meta-analysis of the
relationship between fibrinogen and coronary heart disease (4018 CHD cases in 18 studies), comparison of individuals in the top third with those in the bottom third of baseline measurements yielded a combined risk ratio of 1.8 (95% CI 1.6-2.0), associated with a difference in long-term usual mean fibrinogen levels of 2.9 $\mu$mol/L (0.1g/dl) [194]. Adjustment for other risk factors (serum lipids, alcohol consumption, body mass index, history of diabetes etc) had little effect on this relationship. In Kannel’s analysis of data from the Framingham Study, the independent contribution of fibrinogen to cardiovascular disease risk was greater than that of cigarette smoking [195].

Epidemiological studies show a strong predictive association of individual fibrinogen measurements and risk of cardiovascular disease. Longitudinal studies have indicated that a single fibrinogen measurement predicted fatal and non-fatal cardiovascular events as much as 16 years later [196]. In men in the Northwick Park Heart Study [185] an elevation of one standard deviation (about 0.6g/L) was associated with an 84% increase in the risk of cardiovascular disease within the next five years.

**Other diseases involving atherosclerosis**

Elevated fibrinogen has also been associated with an increased risk of other vascular diseases including cerebrovascular accidents, peripheral vascular disease, venous thrombosis and progressive arterial narrowing [191, 195]. Elevated levels of plasma fibrinogen due to chronic inflammation and activation of the acute phase response have been postulated as a vital link in the association of chronic dental disease with increased risk of cardiovascular disease [197].
Fibrinogen may be causally involved in the production of atherosclerotic lesions. Atherosclerotic lesions are rich in fibrinogen and it is thought that fibrinogen may directly enhance the progression of atherosclerosis, by its conversion to fibrin [193, 198]. However, further research is required to confirm a causal role for fibrinogen in the production of atherosclerotic lesions.

Even if not directly involved in atherogenesis, fibrinogen may contribute to vascular disease by exacerbating the effects of prevalent atherosclerosis. Fibrinogen may promote platelet aggregability [199] and in response to rupture of an atheromatous plaque, fibrinogen is part of the ensuing coagulation response. The thrombus formed may be large enough, in a coronary vessel, to cause myocardial ischaemia. Fibrinogen and plasma lipoproteins are key determinants of plasma viscosity [193, 200], so that elevated levels of fibrinogen may decrease blood flow, resulting in ischaemia if blood vessels are already stenotic.

But elevated fibrinogen levels may also be a consequence of atherosclerosis, which may be viewed as a chronic inflammatory response to endothelial damage. In support of the non-causal hypothesis of the association of elevated fibrinogen and cardiovascular disease, van der Bom showed that while increased fibrinogen was associated with obesity, adverse lipoprotein profile and an increased risk of myocardial ischemia, increased fibrinogen due to genetic causes was not associated with a similar increase in risk [192]. Increased fibrinogen may be a marker of something else that is causing the increased vascular disease. Mirshahi suggests that
secretion of cytokines may simultaneously increase fibrinogen synthesis and induce vascular modifications leading to atherothrombosis [201].

**Interleukin-6 (IL-6) – a link between stress and fibrinogen?**

IL-6 is secreted from activated macrophages, lymphocytes and fibroblasts after activation by IL-1 and TNF-α in the acute phase response [202]. Among other actions, it stimulates hepatic release of fibrinogen.

Il-6 may also be elevated during the stress response via catecholamine-mediated release from adipose tissue. In healthy adults systemic concentrations of IL-6 are increased in the obese and as much as one third of total circulating IL-6 may originate from adipose tissue [202]. IL-6 in turn has stimulatory effects on the HPA, increasing hypothalamic secretion of CRH and responsiveness of both the anterior pituitary release of ACTH and adrenal cortical secretion of cortisol. Yudkin believes that psychosocial stress may increase circulating levels of IL-6, which through stimulation of the HPA axis, results in a tendency to central obesity, insulin resistance and dyslipidemia with increased visceral adipose tissue mass further increasing levels of IL-6 and fibrinogen [202].

Obesity and smoking may resemble a low-grade persistent inflammatory state. Consequent elevation of IL-6 levels and chronic activation of the acute phase response may explain the association of obesity and smoking with elevated levels of fibrinogen [202].
3.8.3 Measurement issues

Fibrinogen has a half-life of 3-6 days, and levels are very responsive to activation of the acute phase response, resulting in considerable intraindividual variation. Several studies have quantified this with repeat measurements. The results range from intraindividual variation accounting for between 12% and 67% of the total variation [190, 203-206]. Values for the correlation between two measurements range from an intraclass correlation of 0.49 (on the basis of 127 individuals who had samples drawn one year apart; Pearson correlation = 0.39 (p<0.0001)) [198] to Pearson correlations of 0.45 (for fibrinogen levels over 1 year among angina pectoris patients) [207] and 0.54 (over 6-7 years among employed men) [208]. Results of a meta analysis return a correlation coefficient between two measurements taken some years apart of 0.6 (range 0.5-0.7) [194].

Circadian rhythm

There are conflicting reports in the literature regarding the presence of a circadian rhythm in levels of plasma fibrinogen. Bremner et al report a significant daily pattern with maximum levels in early to mid morning, based on a study of 11 men ranging in age from 46 to 72 years [209], although there is considerable variability in individual acrophases. However, in a large study in which different groups of individuals were sampled at hourly intervals during the day, no circadian pattern was evident [210].

Seasonal variation

Seasonal variation in cardiovascular mortality has been linked to seasonal changes in fibrinogen level. Seasonal variation in plasma fibrinogen may only be apparent with
some methods of measurement. Two studies have shown significant seasonal variation in fibrinogen levels using immunonephelometry [211, 212]. Maximal levels of fibrinogen occurred in spring (maximum variation of 0.32 g/L). However, measurement of the same samples from one of these studies [211], by the clotting method of Clauss, showed a small but non-significant seasonal variation, with maximum fibrinogen levels in summer. The discrepancy between these two methods was explained by interindividual and intraindividual qualitative differences in fibrinogen and the different sensitivities of different assays to various fibrinogen forms. Using the prothrombin time method, van der Bom found that fibrinogen levels were considerably higher in late winter/early spring, particularly in the elderly. The level of seasonal difference was age dependent with 0.34 g/L (95% CI 0.29-0.39) in the over 75s and 0.29 (0.24, 0.35) in the 55-75 year old group. This variation did not appear to be temperature dependent [213].

3.8.4 Population distribution

Age and Gender

Plasma levels of fibrinogen increase with increasing age in men and in postmenopausal women, but not in premenopausal women [189, 193, 194, 196, 203, 214, 215]. Postmenopausal women had significantly higher levels of fibrinogen than premenopausal women [200, 216]. Over six years of follow-up in 549 women and 440 men, mean fibrinogen increased by 26 mg/dl (0.26 g/L) with the greatest increase in older subjects [217]. Age–adjusted fibrinogen levels are higher in women than men at all ages [216, 219].
### Table 3.1 Seasonal variation of fibrinogen using different methods of analysis.

<table>
<thead>
<tr>
<th>Reference .</th>
<th>No. subjects</th>
<th>Age of subjects</th>
<th>Method of analysis</th>
<th>Seasonal variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Van der Bom (1997) [213]</td>
<td>2325</td>
<td>&gt;55</td>
<td>Prothrombin time derived</td>
<td>Higher in Mar/April &gt;75yo 0.43g/L; 55-75yo 0.29 g/L (Rotterdam) Significant</td>
</tr>
<tr>
<td>Frohlich (1997) [211]</td>
<td>16</td>
<td>20-41</td>
<td>Immuno-nephelometry</td>
<td>Highest in April (Germany): 0.32 p&lt;0.001</td>
</tr>
<tr>
<td>Frohlich (1997) [211]</td>
<td>16</td>
<td>20-41</td>
<td>Clotting method of Clauss</td>
<td>Highest in July (Germany): 0.09; non-significant</td>
</tr>
<tr>
<td>Ma (1999) [193]</td>
<td>400</td>
<td>40-84</td>
<td>Method of Clauss</td>
<td>No significant seasonal variation</td>
</tr>
<tr>
<td>Pitsavos (1998) [212]</td>
<td>2009</td>
<td>18-34</td>
<td>Nephelometry</td>
<td>Seasonal variation – no specific information supplied</td>
</tr>
</tbody>
</table>

### Genetics

Genetic effects may determine 30-50% of interindividual variability in plasma fibrinogen levels and may affect the response of fibrinogen levels to environmental factors [190]. Genetic studies indicate that carriers of the A allele, (approximately 20% of the population), have, on average, 7% to 10% higher fibrinogen levels than those with other genotypes [185]. Smokers with the A-allele experience a greater rise in fibrinogen than those with a different allele.

### 3.8.5 Behavioural variation

#### Diet and Drugs

High levels of fish oil and dietary fibre in the diet, and the use of several drugs (platelet aggregation inhibiting drugs such as ticlopidine and lipid lowering fibrates) may lower levels of plasma fibrinogen [190]. Oral contraceptive use is associated with increased plasma fibrinogen levels [220], while hormone replacement therapy
can impede the elevation of fibrinogen that is observed in post-menopausal women [200].

**Smoking and alcohol**

Plasma fibrinogen levels are positively associated with smoking [189, 190, 193, 203, 216], in smokers consuming more than 10 cigarettes per day [221], and negatively associated with moderate alcohol intake [199, 203, 212]. Cessation of chronic smoking is associated with a marked fall in plasma fibrinogen with levels that remain intermediate between smokers and non-smokers for several years [183, 222].

**Physical activity**

Plasma fibrinogen level is inversely associated with reported leisure-time exercise in some studies, [190, 216] while others find that this relationship is no longer significant after adjustment for possible confounding factors [215]. However, physiological assessment of fitness (maximal oxygen uptake) indicated that those who were most fit had a lower level of fibrinogen, and this relationship persisted even after adjustment for possible confounding factors [215].

Ernst reviewed the relationship between physical activity and fibrinogen levels in longitudinal studies and found evidence of a reduction of about 0.4 g/L with physical activity [223]. Lee suggested that the relationship between activity and fibrinogen could be largely explained by smoking [224], while Connelly found that those who were vigorously active had lower fibrinogen levels, even after adjustment for age, smoking, alcohol, BMI and occupation [225].
3.8.6 Psychosocial and SES variation

A summary of the literature examining the association of psychosocial factors and SES with plasma fibrinogen is presented in Table 3.2. This literature is covered in greater detail, in the context of my own findings, in the Discussion, chapter 6.

### Table 3.2 Summary of SES and psychosocial variation in plasma fibrinogen

<table>
<thead>
<tr>
<th></th>
<th>Direction of effect</th>
<th>Consistency of findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Childhood SES</strong></td>
<td>Inverse association</td>
<td>Few studies, but generally consistent</td>
<td>[196]</td>
</tr>
<tr>
<td><strong>Adult SES</strong></td>
<td>Inverse association</td>
<td>Some consistency; few studies indicate association disappears after adjustment for lifestyle factors, eg smoking.</td>
<td>[182, 196, 200, 218, 226-228]</td>
</tr>
<tr>
<td><strong>Acute stress</strong></td>
<td>Positive association</td>
<td>Five out of seven studies show a positive association.</td>
<td>[182, 229, 230]</td>
</tr>
<tr>
<td><strong>Chronic stress</strong></td>
<td>Positive association</td>
<td>Consistent finding for job stress.</td>
<td>[78, 182, 196, 199, 231, 228, 232]</td>
</tr>
<tr>
<td><strong>Social network/participation</strong></td>
<td>Inverse association</td>
<td>Few studies</td>
<td>[227]</td>
</tr>
<tr>
<td><strong>Social support</strong></td>
<td>Positive association</td>
<td>Few studies</td>
<td>[227]</td>
</tr>
</tbody>
</table>

3.8.7 Association with other cardiovascular risk factors

Plasma fibrinogen levels are positively associated with body mass index [189, 190, 203, 212, 215, 216], LDL cholesterol [189, 190, 216], waist-hip ratio [189, 222, 233], blood leukocyte count [222], history of hypertension [189, 193], and triglyceride level [189] and negatively associated with HDL cholesterol [189, 190, 193, 216, 234] in men and women. In women, obesity, total cholesterol, and systolic blood pressure are positively associated with plasma fibrinogen, while in men, height is negatively correlated with plasma fibrinogen [234].
Plasma fibrinogen concentration is positively correlated with the number of cardiac symptoms experienced in the previous fortnight [196], and with a positive family history of coronary artery disease [216, 221]. In longitudinal studies, fibrinogen increased more in those with, or who developed, diabetes and those whose plasma HDL cholesterol or triglycerides decreased [217].

Margaglione suggests that age and lifestyle factors may account for about 30% of the interindividual variation of plasma fibrinogen levels (with intraindividual variation accounting for up to 25% of the total variability in plasma fibrinogen in the same study) [221].

### 3.8.8 Conclusion

There are plausible biological links between activation of the stress response, and elevation of plasma levels of fibrinogen, via IL-6 and activation of the acute phase response. There is strong empirical evidence that plasma fibrinogen levels predict later coronary heart disease. Fibrinogen is commonly measured, requires no special sample preparation and has a well-defined population distribution. It thus fulfils all of Kelly’s criteria for a marker of the chronic stress response, except that it does not have a long half-life and there is thus considerable intraindividual variability. A single measurement on an individual may provide little indication of the level of chronic stress, although measures averaged over a group may be useful in delineating group associations between stress and SES.
3.9 Glycated haemoglobin (HbA\textsubscript{1c})

3.9.1 Physiology

Human haemoglobin is primarily responsible for oxygen transport by erythrocytes in blood. Haemoglobin is a tetramer consisting of two pairs of entwined and folded polypeptides chains. Several different types of polypeptides, including α, β, γ, and δ chains are found in human red cells, covalently linked to a heme group [235]. In normal adults about 97% of red cell haemoglobin is HbA (α\textsubscript{2}β\textsubscript{2}), which can be separated on cation exchange resin to give haemoglobin fractions designated as HbA\textsubscript{0}, HbA\textsubscript{1}, HbA\textsubscript{2} and HbA\textsubscript{3} [236]. HbA\textsubscript{1} can be further charge separated into HbA\textsubscript{1a1}, HbA\textsubscript{1a2}, HbA\textsubscript{1b} and HbA\textsubscript{1c} [237].

HbA\textsubscript{1c} is created when glucose condenses with a free amino group of valine or lysine on a β chain of haemoglobin to form a Schiff base (pre-A\textsubscript{1c}), which then undergoes rearrangement to form HbA\textsubscript{1c}. The formation of pre-A\textsubscript{1c} is reversible, while the formation of HbA\textsubscript{1c} is irreversible [235]. Since the formation of HbA\textsubscript{1c} is non-enzymatic, the process is termed glycation and HbA\textsubscript{1c} is commonly called glycated haemoglobin, although strictly speaking there are a number of different glycated haemoglobins [237].

Formation of HbA\textsubscript{1c} occurs over the life-course of the red blood cell (~120 days), but recent glycaemia probably has the largest influence on measured HbA\textsubscript{1c}. It is likely that 50% of glycated haemoglobin is formed in the month before sampling, 25% in the month before that, and the remaining 25% in months two to four prior to sampling.
Thus the amount of HbA1c in blood reflects a time-averaged blood glucose concentration over the previous four months [238].

As has been noted earlier, following perception of a stressor the HPA and the SAM are activated, with subsequent rapid elevation of blood glucose level. Repeated elevations of blood glucose, following chronic or repeated acute stress responses could be expected to cause an increase in the level of HbA1c.

### 3.9.2 Association with disease

HbA1c measurement was developed for use in the monitoring of blood glucose levels in diabetics, but has been little studied in non-diabetic populations. In a cross-sectional examination of postmortem atherosclerosis and glycated haemoglobin in young non-diabetic persons dying of external causes, McGill found that there was evidence of extensive early atherosclerosis in those with glycated haemoglobin levels greater than 8%. There was no association with atherosclerosis at lower levels of HbA1c [239].

In a case-control study as part of the Atherosclerosis Risk in Communities Study (ARIC) study, after adjustment for other risk factors, a 1% increment in glycated haemoglobin level was associated with 1.77 greater odds of having carotid intimal-medial thickening, a measure of asymptomatic atherosclerosis. However, it was uncertain whether the association was linear, or whether there was a threshold, possibly within the normoglycemic range [240]. Other studies have also reported an
association between the level of glycated haemoglobin and symptomatic cardiovascular disease in non-diabetic individuals (cited in [240]).

Glycated haemoglobin may not be only a marker of diabetes or atherosclerosis, but part of the disease process itself. HbA\textsubscript{1} is not the only protein to undergo glycation in the presence of glucose and other sugars. Over time non-enzymatic glycation of proteins leads to the formation of irreversible terminal products, the advanced glycation end-products (AGEs). These accumulate in long-lived proteins such as collagen. There is now extensive evidence that AGEs play an important role in the pathogenesis of diabetic complications, including nephropathy and retinopathy by a variety of mechanisms including DNA mutation, generation of oxygen free radicals and alteration of tissue immunogenicity [241].

3.9.3 Measurement issues

In general HbA\textsubscript{1c} is expressed as a percentage of total haemoglobin, but there are a number of issues in its measurement.

**Haemoglobin variants**

As well as the HbA group of haemoglobin fractions, there are more than 700 other haemoglobin variants, which may be present in human red cells. At least half of these are clinically silent; others including HbS, HbC, HbD, and HbE may cause various levels of sickle cell anaemia, while variants such as HbF (\(\alpha_2\gamma_2\)) or fetal haemoglobin are commonly present but in small amounts in adult humans. A number of these
abnormal haemoglobins can interfere with the measurement of HbA1c, either by
giving falsely high or low results, or by making the measurement impossible.

Measurement methods
There are currently about twenty different methods of measuring glycated
haemoglobin, using four different principles – affinity chromatography,
imunoagglutination, electrophoresis and high performance liquid chromatography
[238, 242]. Unfortunately, different techniques may measure slightly different
glycated products, use different units for reporting results, and can produce different
values for the same blood sample. Indeed, different laboratories using the same assay
may not produce similar results. Efforts are underway to standardise the analysis and
reporting terminology [242].

Individual variability
There is strong intraindividual stability of the measure but wide inter-individual
variability [243].

Seasonal variation
Research suggests a seasonal peak in early spring and a nadir in early autumn [244,
245].

3.9.4 Population distribution
In a random sample of Victorians aged 40 years and older, (as part of the Melbourne
Visual Impairment Project) 3% of the non-diabetic study population had an elevated
level of HbA1c [246].
Age and Sex

Individual concentrations increase with age at approximately 1% per year [154, 247]. While some studies suggest that men tend to have higher levels of glycated haemoglobin than women, at all ages with no effect of menopausal status [248], others indicate no gender difference in non-diabetic populations [247, 249].

3.9.5 Behavioural variation

Physical activity

Poorer diabetic control is associated with low levels of physical activity [250] as well as lower socioeconomic status and lower educational level. While this is reflected in a higher HbA₁c, it is not possible to understand the effect of physical activity on HbA₁c without taking account of the confounding effect of socioeconomic status and diabetic status.

Smoking

Current smokers have higher HbA₁c levels than ex-smokers or non-smokers [248].

3.9.6 Psychosocial and SES variation

A summary of the literature outlining the psychosocial and SES variation in glycated haemoglobin is presented in Table 3.3. These findings are covered in greater detail, in the context of my own findings, in the Discussion, Chapter 6.
Table 3.3 Summary of SES and psychosocial variation in glycated haemoglobin.

<table>
<thead>
<tr>
<th>SES measure</th>
<th>Direction of effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult SES</td>
<td>null</td>
<td>Few studies in general population; no convincing association [250-252]</td>
</tr>
<tr>
<td>Acute stress</td>
<td>Positive association</td>
<td>Examination stress. No association with other psychological factors. [244]</td>
</tr>
<tr>
<td>Chronic stress</td>
<td>Inferred stress in Indigenous populations [251]</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>Positive association</td>
<td>Single study looking at anxiety levels in relatives of cancer patients [247]</td>
</tr>
<tr>
<td>Social support</td>
<td>null</td>
<td>Few studies in non-diabetic population [244]</td>
</tr>
</tbody>
</table>

3.9.7 Association with other risk factors for disease

Few researchers have examined the association between glycated haemoglobin and risk factors for disease in non-diabetic populations. In a Japanese study, levels of glycated haemoglobin were significantly correlated with levels of fibrinogen and factor VII in men and with BMI and total cholesterol in women in multivariate analysis [248].

3.9.8 Conclusion

Glycated haemoglobin is plausibly increased as a result of repeated or prolonged activation of the stress response, via elevation of blood glucose under control of the SAM and the HPA. There is little evidence that elevation of HbA\textsubscript{1c} within the normal range is predictive of later disease, although mild elevations may be associated with subclinical atherosclerosis. Glycated haemoglobin has a long half-life, a well-defined demographic distribution, is commonly measured and requires no particular sample preparation. It has readily available reference standards, but there are problems with
the interlaboratory correlation of measurements. There is a good fit with Kelly’s criteria for use of HbA1c as a marker of the chronic stress response.

### 3.10 Waist–hip ratio

#### 3.10.1 Physiology

Waist – hip ratio (WHR) is simply the waist circumference divided by the hip circumference. It is measure of fat distribution and visceral fat mass, unlike body mass index (BMI = weight/height$^2$), which is a measure of generalised obesity, measuring the sum of fat mass and fat free mass, regardless of the distribution. It seems likely that BMI is dependent on the regulation of energy balance via the HPA and leptin resistance, whereas localisation of body fat is probably regulated by other factors, such as endocrine perturbations [253].

There is high (but not perfect) correlation between BMI and WHR [254, 255] and increased health risks are associated with abnormalities of each, independently.

Figure 3.3 indicates the health risk associated with different body profiles. In general, low BMI (leanness) is associated with better health than high BMI, but being lean and having greater central fat is a particularly unhealthy profile, which predicts premature mortality [254].
In the general population, around 65-70% of body fat is subcutaneous, 20% intra-abdominal and 12% intra-muscular [257]. Premenopausal women preferentially deposit fat in the gluteofemoral region and carry on average only 8% of their total body fat mass in the abdominal region [258]. Increased abdominal adiposity may be due to increased intra-abdominal fat or increased subcutaneous fat (lying between the abdominal muscles and the skin). Approximately 80% of intra-abdominal fat is visceral fat, while 20% is retroperitoneal [257] and it appears to be increased visceral fat that is particularly associated with poorer health outcomes [259].
Pathogenesis of central fat deposition

Fat deposition is a complex process controlled by brain neuropeptides, leptin, the SNS and the HPA. Obesity in turn affects the SNS and the HPA axis through secretion of IL-6 from adipose tissue.

During puberty, under the control of androgens and estrogens, characteristic male and female fat distribution is established. Males develop a more central fat distribution, whereas females tend to store more fat peripherally, [260, 261] although the preferential site of fat deposition is determined by genetic and environmental factors [254, 260]. Estimates of the contribution of genetic factors to the variation in fat distribution vary from around 25% [260] to 50% [262] and these genetic factors probably work through alterations in the HPA and ANS.

Perhaps the most striking example of central body fat deposition is that seen in Cushing’s syndrome, the result of diseases that cause elevated levels of cortisol. This typical pattern of fat deposition associated with cortisol excess has led to considerable research investigating the role of cortisol and the HPA axis in the development of central adiposity in non-Cushingoid individuals. Such people display increased excretion of (urinary) free cortisol that is proportional to WHR, and blunting of dexamethasone suppression (a test of an intact cortisol negative feedback on ACTH), with depressed levels of circulating sex steroids and growth hormone [263]. The HPA appears to be hyper-responsive and it is hypothesized that increased levels of cortisol stimulate the increased deposition of body fat. Sex steroids and growth hormone (GH), which have opposite effects on fat deposition, are depressed, further favoring
net fat deposition [263]. Indeed, GH and female sex hormones not only mobilize fat, but they cause it to be stored peripherally (at the hips) rather than centrally [254].

Björntorp theorizes that stress-induced abnormalities in the HPA are causative of central fat deposition. The defeat response to stress is characterized by elevated secretion of cortisol in response to ACTH stimulation [264] and decreased secretion of growth hormone and sex steroid hormones [263]. In humans with central adiposity, there is physiological evidence of both the fight/flight and defeat responses to stress, with hypertension, high haemoglobin concentration and increased plasma free fatty acids (FFAs) [265].

Similar outcomes have been observed in rats and in non-human primates under chronically stressful conditions. Studies in cynomolgus monkeys indicate that socially subordinate animals who are the recipients of constant aggression by dominant monkeys experience a defeat reaction associated with this chronic stressor exposure, with activation of the HPA and accumulation of excess visceral adipose tissue in a dose-dependent manner [163, 254, 261, 266-268]. There is concomitant development of insulin resistance, dyslipidemia, hypertension, impaired glucose tolerance and early signs of coronary atherosclerosis [162].

In humans, chronic over-activity of the SNS may manifest as essential hypertension [263], while chronic activation of the HPA axis results in the loss of the normal diurnal rhythm of cortisol excretion, with lower morning values and blunted responses to stressor exposure [163, 269]. In association with blunting of the HPA

130
axis, the central glucocorticoid receptors (GCRs) may become atrophied and less dense, with diminished cortisol feedback inhibition of CRH and ACTH and an abnormal dexamethasone suppression test. The SNS may become overactive in compensation [163]. Consequently, adults with central fat distribution may have greater cardiovascular reactivity to stress (such as blood pressure reactivity) than those with peripherally distributed fat [254].

In adipocytes, glucocorticoids (in the presence of insulin) inhibit the fat-releasing effects of insulin (by inhibiting hormone sensitive lipase) [163], and promote fat storage by enhancing the activity of lipoprotein lipase [270]. The net result is an ever-increasing accumulation of fat and enlargement of adipocytes. Visceral fat is morphologically different from peripheral fat with higher levels of GCRs (fourfold higher in intraabdominal fat than in subcutaneous fat [270]), greater blood flow and denser innervation. Thus cortisol acts via the GCRs preferentially at visceral sites to increase fat deposition [166, 263, 271-273]. This is summarized in Box 3.4.

**Box 3.4. Cortisol effects on visceral fat**

- Binding to glucocorticoid receptors
  - (High density of receptors)
  - Lipoprotein lipase activated $\rightarrow$ Triglycerides accumulated
  - (Gene transcription & enzyme stabilization)
  - Lipid mobilisation inhibited $\rightarrow$ Triglycerides retained
  - (Insulin required) $\rightarrow$ [163]
The reproductive system is inhibited at all levels by components of the HPA axis: CRH suppresses hypothalamic LHRH, while cortisol inhibits the actions of pituitary and gonadal sex hormones on target tissues [273]. Consequently, women with central obesity may have a higher incidence of oligomenorrhea, elevated free testosterone levels [163], and low levels of sex hormone binding globulin [261]. Men with elevated WHR are prone to relative hypogonadism and abnormally low levels of testosterone [273].

Growth hormone (GH) and the sex hormones have opposite effects to cortisol on lipid accumulation, inhibiting lipoprotein lipase and stimulating lipolysis [163]. Thus the prolonged combination of elevated cortisol levels and low secretions of sex steroids and GH (as in the defeat response to stress) will tend to favour the accumulation of body fat, with preferential accumulation in intraabdominal visceral depots [163], insulin resistance and ultimately the metabolic syndrome [274]. Figure 3.4 summarizes the pathophysiology of central obesity.

Other hypotheses regarding the pathogenesis of central fat distribution include a primary adrenal [261], hypothalamic or pituitary [166] abnormality with hyper-responsiveness of the HPA and excessive secretion of CRH and cortisol. Depression of the normal negative feedback mechanism could be the result of downregulation of GCR due to HPA hyperactivity or a genetic polymorphism at the level of the coding sequence of the GCR gene [166].
SNS: sympathetic nervous system
FFA: free fatty acids
VLDL: very low density lipoproteins
CVD: cardiovascular disease
ACTH: adrenocorticotrophin hormone
FSH/LH: follicle stimulating hormone/luteinizing hormone
3.10.2 Association with disease or risk factors for disease

Why is the distribution of body fat important?

Vague (1956) was the first to propose that the level of body fat was less important to health than the distribution of this fat, following studies of health outcomes in obese mice (cited in [259]). Subsequent studies have confirmed that centrally deposited fat is a greater risk factor for a number of diseases, including cardiovascular disease and diabetes, than peripherally deposited fat [162].

WHR and the metabolic syndrome

The metabolic syndrome is a cluster of signs including insulin resistance, dyslipidemia (elevated cholesterol and triglycerides, elevated LDL and depressed HDL cholesterol), glucose intolerance, hypertension and a hypercoagulable state. Individuals with elements of the metabolic syndrome are at increased risk of cardiovascular disease. Elevated WHR is an important feature of the metabolic syndrome and may be the driving force behind the associated abnormalities [257, 275].

WHR and cardiovascular disease

In cross-sectional studies, elevated WHR and excessive visceral fat or central body fat distribution are associated with hypertension [276, 277], peripheral arterial disease, cardiovascular disease [239, 259, 278, 279] and cerebrovascular disease [261, 280] in men and women [259, 263, 281, 282]. In many studies this association is stronger than the association between these diseases and BMI and persists after
adjustment for total cholesterol level, blood pressure, diabetes, BMI, smoking, alcohol consumption, education and race [282-285].

Increased central fat deposition is also associated with other measures of cardiovascular risk, such as increased levels of fibrinogen, total cholesterol, decreased HDL cholesterol and elevated triglycerides [257, 278, 279, 286-288]. The association between central adiposity and disease appears to be dose-related such that higher WHR predicts higher disease risk and this is independent of sex, age and race [256, 285, 289].

McGill noted that visceral adiposity at postmortem, in young persons dying of external causes, was associated with increased vascular fatty streaks and atherosclerosis in the coronary artery [239].

**Association with diabetes mellitus**

There is a strong association between central fat deposition and (non-insulin dependent) diabetes mellitus Type 2, in adults [261, 290, 291]. In the Iowa Women’s Health Study, women simultaneously in the highest quintiles of BMI and WHR had a relative risk (RR) of (self-reported) incidence of diabetes of 29 (95% CI 18-46) compared to women in the lowest combined quintiles. Even women in the lowest quintile for BMI had increased risk of developing diabetes if they had elevated WHR (RR = 5.1, 95% CI 3.9 - 6.8) [289].
Association with malignancy

There is a positive association of central fat tendency with breast and endometrial cancers in women [259, 261] with a relative risk (RR) for developing breast cancer of 1.3 (95% CI 1.1 - 1.5) and for endometrial cancer RR = 2.0 (95% CI 1.4 - 2.8) in women in the highest quintile of WHR compared to the lowest [289]. Among women with a family history of breast cancer, the relative risk for developing breast cancer for those in the highest quintile of WHR was 3.24 [292]. Hyperinsulinemia is commonly seen in association with central adiposity [293] and insulin is a known growth-promoting hormone for normal breast epithelium and human breast cancer cells. In addition, visceral adipose tissue synthesizes significant amounts of insulin-like growth factors, which may facilitate the response of breast cancer cells to estradiol [292].

Association with other diseases

Increased central adiposity is associated with sleep apnoea (in men) [294] and renal impairment [295] even in those with normal BMI. WHR predicts total mortality, as well as mortality from the specific diseases already outlined [289]. The relative risk for stroke in a large study of male health professionals was 2.33 (95% CI 1.25-4.37) in the highest quintile of WHR compared to the lowest [280].

WHR and BMI are also associated with psychiatric ill-health, including use of psychopharmacological drugs, personality disorders, depression, anxiety and psychosomatic disease, but this relationship appears to be stronger for WHR in women than men [261, 296, 297]. Non-obese depressed women had higher cortisol
levels and a twofold increase in intra-abdominal fat compared to controls (non-depressed) [270].

Obesity is increasing in the populations of developed countries over time, but the increase in central obesity appears to be more marked than the increase in generalized obesity [298].

3.10.3 Measurement issues

A number of different measurements have been used to evaluate central fat deposition, including waist - hip ratio, waist circumference and waist to height ratio [276, 279]. Waist circumference is often more highly correlated with BMI than with WHR and may reflect both general and abdominal obesity, thus adding little further information beyond that contributed by BMI [276, 289]. WHR appears to predict disease in adults better than other simple measurements [259].

Waist and hip measurements may each provide different health information [261] and it seems likely that it is the combination of increased waist circumference with relatively decreased hip circumference that is predictive of disease, rather than just increased waist circumference [299]. Hip circumference includes the measurement of the large muscles in the gluteal region, and muscle tissue is a major regulator of systemic insulin sensitivity [160]. A small hip circumference, indicating a small muscle mass may mean less quantity of this important tissue [160]. The combination of high waist circumference (with excessive visceral adipose tissue and release of FFAs into the circulation) and low hip circumference (with lower gluteal muscle
mass), may be particularly important in relation to insulin resistance. Thus, while waist circumference may be an important indicator of increased cardiovascular disease (CVD) risk, both hip and waist circumference may carry information for insulin resistance.

WHR is highly correlated with more advanced methods of determining visceral fat mass, e.g. magnetic resonance imaging, dual energy X-ray absorptiometry and CT scan [259, 289, 300].

There is considerable variability in the position of the measurements taken. Waist measurements have been taken at the level of the umbilicus [267], midway between the costal margin and the iliac crest [282], 2.5 cm above the umbilicus [289], at the mid-level between the processus xiphoideus and the umbilicus [233] and at the smallest diameter between the rib cage and the iliac crest [274]. Hip measurements have been taken at the level of greater trochanters [267], the largest girth between the waist and thigh [274], the widest point between the hip and the buttock [282] or at the level of maximal protrusion of the gluteal muscles [276, 289].

For consistency all subjects must be similarly clothed although there is a high correlation ($r = 0.994$) between measurements taken over clothing and those taken over skin [298]. Some researchers have measured the tension of the measuring tape to ensure consistency in technique [287]. Despite the variation in measurement technique, the associations with disease are robust and were apparent even when participants self-measured using a paper tape [262, 296]. Comparisons of different
versions of the WHR measurement suggest that they measure disease risk similarly [301].

Upper limits of WHR vary by gender and in different populations. A survey of Australian populations in 1993 returned a mean of 0.89 for men and 0.76 for women aged 40-44 years [301]. Increased CVD risk may be present when WHR exceeds 0.85 [259].

### 3.10.4 Population distribution

**Age and Gender**

Central fat distribution increases with age and is correlated with the level of decline of the sex hormones. Higher WHR is more common in males and post-menopausal women and is correlated with parity [302].

**Genetics and intrauterine effects**

The greatest risk for developing abdominal obesity and insulin resistance occurs in children who are born small but gain weight rapidly between ages 4 to 7 [257, 303]. Higher birth weight is associated with higher adult BMI, while maternal and paternal obesity predict childhood obesity in their children [303].
3.10.5 **Behavioural variation**

**Smoking**

Most studies indicate that central body fat tendency is more prevalent among smokers [259, 265, 286, 289, 297, 304], possibly associated with hypothalamic arousal, hypercortisolemia and depressed levels of sex hormones similar to that seen in the defeat response to stress. Male smokers tend to have increased WHR but decreased BMI, that is, they are leaner than non-smokers but tend to deposit fat centrally [259, 301].

**Alcohol**

Alcohol intake has an inconsistent association with WHR [259, 301, 302].

**Physical activity**

Physical activity is negatively associated with both abdominal adiposity and BMI in a dose-response manner [301, 305], although Visser found that this association was restricted to intensive exercise training (but not low or moderate intensity exercise) [305].

3.10.6 **Psychosocial and SES variation**

The literature examining associations between psychosocial factors and WHR and the SES variation in WHR is summarized in Table 3.4. These findings are covered in greater detail, in the context of my own findings, in the Discussion, Chapter 6.
### Table 3.4 Summary of SES and psychosocial variation in waist-hip ratio.

<table>
<thead>
<tr>
<th></th>
<th>Direction of effect</th>
<th>Findings</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Childhood SES</td>
<td>Inverse association</td>
<td>Highly consistent</td>
<td>[281, 286, 306-310]</td>
</tr>
<tr>
<td>Adult SES</td>
<td>Inverse association</td>
<td>Consistent. May be strongest with education. 30% of studies report direct relationship in men. Inconsistent results with employment status and for housing. Subjective SES may be more strongly associated than objective indicators, for women.</td>
<td>[150, 226, 256, 265, 287, 290, 297, 301, 304, 308, 311-314]</td>
</tr>
<tr>
<td>Acute stress</td>
<td>Direct association</td>
<td>May be an interaction with BMI in men</td>
<td>[254, 315, 316]</td>
</tr>
<tr>
<td>Chronic stress</td>
<td>Direct association</td>
<td>Strain in social relationships, perceived inequality and constraint, work stress</td>
<td>[254, 263, 310]</td>
</tr>
<tr>
<td>Affiliation and extroversion</td>
<td>Direct association</td>
<td></td>
<td>[297]</td>
</tr>
<tr>
<td>Self-esteem</td>
<td>Inverse association</td>
<td></td>
<td>[254]</td>
</tr>
<tr>
<td>Social support</td>
<td>Inverse association</td>
<td>Lack of social support associated with increased risk of the metabolic syndrome.</td>
<td>[317]</td>
</tr>
</tbody>
</table>

#### 3.10.7 An evolutionary perspective

Vague referred to central fat tendency as an android fat distribution, more commonly seen in males. Gynoid fat distribution, more common in women is characterised by localisation of fat in the thighs and buttocks. Abdominal adipose tissue is metabolically active and relatively easily mobilized to provide energy in time of need, such as in response to stressor exposure, and may therefore have survival value [160]. Fat stores in the gluteofemoral area, more common in premenopausal females, may have evolved primarily as an energy store (during pregnancy and lactation), insulator or to have a reproductive function [256]
3.10.8 Conclusion

WHR is a stable, easily measured, biological marker with strong associations with cardiovascular disease and insulin resistance. There is considerable evidence that central fat deposition may be a consequence of activation of the neuroendocrine stress response, particularly to stressors perceived as being overwhelming.

In addition to an association with the metabolic syndrome and a number of endocrine aberrations, central fat deposition is also associated with adverse behaviours such as smoking, excessive alcohol ingestion, sedentary lifestyle, high daily caloric intake and weight gain during adulthood as well as psychiatric disorders, such as depression and anxiety, peptic ulcers and increased use of stimulants [263, 273]. Bjorntorp suggests that this constellation of behavioural and metabolic abnormalities is the human equivalent of the defeat reaction seen in cynomolgus monkeys [160].

3.11 Neopterin

3.11.1 Introduction

In its simplest conceptualisation, the immune system can be divided into cell mediated immunity (activated T cells stimulate the body’s own germicidal cells to engulf and destroy pathogens) and humoral immunity (plasma cells produce antibodies that bind with pathogens, which can then be destroyed by various means). Activated T cells of the cell-mediated (Th1) and the humoral (Th2) immune systems release a number of mediators called cytokines, with diverse actions throughout the
body. (A more detailed description of the immune system is provided in Appendix A).

While measurement of cytokines may provide a window on the immune system, most have been difficult to measure as they are often labile, bind to target cells and may disappear from the circulation soon after release [318]. Neopterin is a sensitive and stable marker of the cell mediated immune system reflecting the outcome of activation of the system and the net biological effect of release of a mixture of cytokines on the macrophage population in vivo [319, 320].

Neopterin belongs chemically to the family of pteridines. First isolated as pigment from the wings of butterflies (Lepidoptera) by Hopkins in 1889, the name pteridines was coined in 1936 by Wieland and Schopf, from the Greek pteron, meaning wing [321, 322].

In 1963 neopterin was isolated from the larvae of bees and in royal jelly [321, 322] and was first detected in human urine in 1967 [318]. It was subsequently isolated from the urine of patients with malignant tumours and viral diseases in 1979 [318, 323] and observed to accumulate in blood following antigenic stimulation in 1982-3 [318].
3.11.2 Physiology

**Biosynthesis**

Activation of T<sub>H1</sub> type T cells results in the release of interleukin 2 (IL-2) and subsequently interferon gamma (IFN-γ). IFN-γ acts on monocytes/macrophages to stimulate conversion of guanosine triphosphate (GTP) to 7,8 dihydroneopterin (DHN), which may then be oxidized to neopterin [324].

In most human cells, including fibroblasts, endothelial cells and cells of the central nervous system, GTP metabolism results in the formation of tetrahydrobiopterin (BH<sub>4</sub>) and only small amounts of neopterin. BH<sub>4</sub> is an essential electron donor in the hydroxylation of phenylalanine to tyrosine in the liver, and of tyrosine to L-dopa and tryptophan to 5-hydroxy-tryptophan in neuroendocrine tissue synthesizing catecholamines or serotonin [321, 324]. Monocytes/macrophages however, lack the enzyme 6-pyruvoyl-tetrahydropterin synthetase, which converts GTP to BH<sub>4</sub>, and consequently preferentially form DHN and neopterin [321]. Atypical phenylketonuria is caused by defective biosynthesis of tetrahydrobiopterin with accumulation of phenylalanine and neopterin, and deficient production of the neurotransmitters dopamine, noradrenaline and serotonin [318].
Biosynthesis of neopterin from guanosine triphosphate (GTP). PTPS is the enzyme 6-pyruvoyl-tetrahydropterinsynthase. 7,8-dihydroneopterin triphosphate is converted via a magnesium-dependent 6-pyruvoyl-tetrahydropterin synthetase to unstable 6-pyruvoyl-tetrahydropterin, which in turn is converted to 5,6,7,8-tetrahydrobiopterin an inhibitor of GTP-cyclohydrolase I. 5,6,7,8-tetrahydrobiopterin is an electron donor in the hydroxylation of phenylalanine to tyrosine in the liver, tyrosine to L-DOPA and tryptophan to 5-hydroxytryptophan in neuroendocrine tissues synthesizing neurotransmitters such as catecholamines and serotonin. 5,6,7,8-tetrahydrobiopterin is also a cofactor of nitric oxide synthetase [325].
**Stimulants of neopterin release**

The main stimulant of neopterin production is IFN-γ, while IL-2 also provokes neopterin production via induction of IFN-γ and tumour necrosis factor alpha (TNF-α) [324]. Interferon-alpha (IFN-α) can stimulate neopterin production in vitro, but only at a dose 1000-fold higher than the required dose of IFN-γ [321, 323, 324]. While such levels are unlikely in vivo, high local levels of IFN-α may occur in some autoimmune diseases and in AIDS, so that in these situations IFN-α may be responsible for induction of neopterin production [326]. T_{H}1 immunity is inhibited by activation of the T_{H}2 (humoral) immune response, so that neopterin production is suppressed by T_{H}2 cytokines, IL-4 and IL-10 [327].

The interaction between macrophages and activated T lymphocytes is a dynamic one, with each influencing the behaviour of the other. Interleukin-1 (IL-1) acts on T cells to induce IL-2 receptors and to stimulate IL-2 production. IL-2 in turn stimulates T cells to secrete IFN-γ, which activates macrophages to produce large amounts of neopterin [321].

The physiological response to acute stress results in the elevation of IL-1β and TNF-α, and downregulation of IFN-γ [328-330]. The response to acute stress should therefore be associated with a decrease in neopterin levels. The T_{H}1 (cell-mediated) and T_{H}2 (humoral) branches of the immune system are generally counterregulatory, so depression of neopterin levels (as a sign of cell-mediated immunity) fits well with the enhancement of humoral immunity seen with acute stress [331].
On the contrary, chronic stressor exposure may be associated with diminished production of IL-1 β [333], and increased serum neopterin. There are however, contradictory findings of the effects of acute and chronic stress on cell-mediated immunity, depending on the parameters measured for both stress and immunity [334].

**Biological Role**

Neopterin is found only in primates so that it has been hypothesized to be the primate homologue of nitric oxide [335, 336], a substance important as a neurotransmitter (when secreted by neurons), as a vasodilator (when secreted by endothelial cells) and as a cytotoxin to certain cancer cells and bacteria when secreted by white blood cells [337].
**Redox reactions**

Neopterin and 7,8-dihydroneopterin (DHN) coexist in a ratio of approximately 1:2 [318]. At a slightly alkaline pH (pH 7.5), neopterin enhances the activity of powerful oxidising agents such as hydrogen peroxide and chloramine –T [321, 338]. DHN on the other hand is an antioxidant, which scavenges free radicals. Thus neopterin/DHN can both enhance and reduce cytotoxicity by powerful oxidants, depending on the pH and the oxidation state of neopterin. The cytotoxic potential of activated macrophages may be enhanced by neopterin, while intracellular accumulation of DHN may protect the cells from damage by reactive oxygen species (ROS) [321, 325, 332, 339].

**Modulation of macrophage mediated effector mechanism**

Interferon gamma and neopterin are produced in the course of a cellular response to an antigen. Neopterin may act as an endogenous inhibitor of folate synthesis by pathogenic micro-organisms within the macrophage, aiding that cell to destroy such pathogens [321].

**Neurodegeneration**

Neopterin is elevated in the cerebrospinal fluid (CSF) of patients with inflammatory neurological disorders, including AIDS dementia, Lyme disease and multiple sclerosis [340]. Neopterin, DHN and ROS are secreted by infiltrating monocytes/macrophages rather than from neuronal cells [340]. Prolonged production of ROS and their enhancement, e.g. by neopterin, can result in oxidative stress and cellular apoptosis which may play a part in the pathogenesis of neurodegenerative diseases [340, 341]. Increased concentrations of neopterin derivatives are also seen in
some patients with neurodegenerative disorders such as Alzheimer’s disease and Huntingdon’s disease [341].

**Tumour angiogenesis**

By disturbing the cellular redox state in the tumour microenvironment neopterin may promote tumour growth and, via promotion of angiogenesis, promote metastatic spread [325, 342].

**Inhibition of erythropoiesis**

Neopterin inhibits production of erythropoietin and may play a role in the generation of anaemia commonly seen in diseases involving chronic inflammation or malignancy [325, 336].

**Atherosclerosis generation**

Neopterin has effects on lipid metabolism in the liver [343] and has multiple roles in atherogenesis by promoting cholesterol precipitation [344], vascular smooth muscle cell proliferation [321, 345] and upregulation of IL-6 and TNF-α [346]. There is an overall increase in inflammatory tone in the vascular wall [346], and activated macrophages produce proteases that can weaken the structure of the plaque, causing rupture [325, 346]. Serum neopterin levels are correlated with the extent of carotid atherosclerosis and neopterin levels may be elevated in unstable coronary artery disease [325].
Interaction with UVA

Neopterin appears to enhance UVA induced cytotoxicity in mouse melanoma cells [321, 336].

3.11.3 Neopterin and disease

Neopterin levels are a direct measure of activation of cell mediated immunity and reflect the level of oxidative stress during an immune response [324]. Neopterin concentration increases rapidly during an immune response, correlates with the intensity of the underlying stimulus and decreases rapidly on cessation of the stimulus [332].

Neopterin concentration is elevated in atypical phenylketonuria and serial measurements allow monitoring of the progress of diseases in which there is activation of cellular immunity – organ and bone marrow transplantation, some malignancies, autoimmune diseases, some infections (particularly viral) and diseases of chronic inflammation [324, 339]. Patients undergoing immunostimulatory treatment show increased levels of neopterin, and neopterin levels decrease when graft rejection is successfully treated by cyclosporin A or corticosteroids [321]. In a number of diseases, higher levels of neopterin are predictive of more extensive disease, worse prognosis and more rapid disease progression and death [324]. Table 3.2 summarizes disease associations with serum neopterin levels.

Concentrations of neopterin are significantly higher in patients with viral infections compared to those with bacterial infections [320], reflecting the greater involvement
of the T<sub>H</sub>1 immune system in viral infections [336]. In common acute viral infections such as measles, mumps, chickenpox, rubella and influenza, elevated neopterin levels are seen at the end of the incubation period prior to the onset of clinical symptoms and reach maximum levels just before specific viral antibodies become detectable [336]. Vaccination with live measles/mumps vaccine causes a similar pattern of neopterin secretion, with declining levels as specific antibodies are produced [321].

Diagnostically neopterin levels may help to distinguish autoimmune disease from non-immune disorders, e.g. rheumatoid arthritis from osteoarthritis, autoimmune thyroiditis and non-autoimmune thyroid disease and infectious and non-infectious hepatitis [318]. Neopterin levels are significantly correlated with diastolic blood pressure (r = 0.13, p<0.0001) and (weakly) with body mass index (r = 0.084, p<0.01) [347].
Table 3.5 Diseases accompanied by changes in neopterin levels

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Variation in neopterin</th>
<th>Higher levels predict worse disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organ transplantation</td>
<td>Higher levels indicative of immunological complications</td>
<td>Yes</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Higher levels reflect more active disease. Helpful in diagnosis to distinguish from osteoarthritis</td>
<td>Yes</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Higher neopterin levels reflect more active disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Crohn’s colitis</td>
<td>In adult and juvenile forms, high neopterin associated with more active disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Linear correlation with anti-gliadin antibodies. Decline with gluten-free diet</td>
<td>Yes</td>
</tr>
<tr>
<td>IDDM</td>
<td>Juvenile (autoimmune) diabetes associated with increased neopterin levels</td>
<td>?</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>Autoimmune type associated with increased neopterin. Useful diagnostically.</td>
<td>?</td>
</tr>
<tr>
<td>SLE</td>
<td>Decrease in those where steroids cause clinical improvement. Higher levels with active disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Slightly elevated CSF (but not urine or serum) levels in active disease, exacerbations and relapse.</td>
<td>No</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>High levels in active disease and slightly raised even in inactive disease</td>
<td>Yes</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>Good correlation between neopterin and disease stage and left ventricular function tests</td>
<td>Yes</td>
</tr>
<tr>
<td>Acute rheumatic fever</td>
<td>Neopterin levels at presentation may be a predictor of valve lesions</td>
<td>?</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>Conflicting results for unstable angina, stable angina. Those with acute myocardial ischaemia or exacerbations of unstable angina have rising neopterin levels</td>
<td>?</td>
</tr>
<tr>
<td>Hematological neoplasms (90%)</td>
<td>Non-Hodgkins lymphoma, chronic lymphocytic leukemia. May be elevated in Hodgkins lymphoma and multiple myeloma</td>
<td>Yes</td>
</tr>
<tr>
<td>Gynaecological malignancies</td>
<td>High incidence of increased neopterin in ovarian cancer (80%). Also seen in carcinoma of the uterine cervix (55%), uterine sarcomas</td>
<td>Yes</td>
</tr>
<tr>
<td>Genito-urinary malignancies</td>
<td>High incidence of increased neopterin in bladder tumours and prostatic carcinoma. Also seen in renal cell carcinoma, testicular cancer</td>
<td>Yes</td>
</tr>
<tr>
<td>Lung cancer (58%)</td>
<td>Increased neopterin often present at diagnosis; no difference in neopterin by histological type</td>
<td>Yes</td>
</tr>
<tr>
<td>Gastrointestinal malignancies</td>
<td>Elevations may be seen with gastric carcinoma, colorectal carcinoma, pancreatic carcinoma, biliary tract carcinoma, hepatocellular carcinoma</td>
<td>In some cancers, not in others</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Occasionally associated with elevated neopterin</td>
<td>Slight</td>
</tr>
<tr>
<td>Malignant melanoma</td>
<td>May be useful for detection of visceral metastases. Neopterin increases precede clinical deterioration</td>
<td>?</td>
</tr>
<tr>
<td>Condition</td>
<td>Description</td>
<td>Indicator</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td>Viral infections</td>
<td>Elevated neopterin in cytomegalovirus and Ebstein Barr virus infections, chickenpox, measles, acute viral hepatitis, chronic viral hepatitis</td>
<td>High levels during acute infection</td>
</tr>
<tr>
<td>Malaria</td>
<td>Almost all patients with acute malaria have elevated neopterin. In P.falciparum infection positively correlated with the degree of parasitemia. Neopterin levels normalize with treatment</td>
<td>Yes</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>Worse disease with hepatosplenic involvement is associated with higher neopterin levels</td>
<td>Yes</td>
</tr>
<tr>
<td>Pulmonary tuberculosis</td>
<td>Extent and activity of infection correlates with neopterin levels</td>
<td>Yes</td>
</tr>
<tr>
<td>Leprosy</td>
<td>Most patients have increased neopterin.</td>
<td>No</td>
</tr>
<tr>
<td>Melioidosis</td>
<td>Elevated levels in patients compared to controls</td>
<td>Yes</td>
</tr>
<tr>
<td>Lyme disease</td>
<td>Normal serum and urine levels of neopterin but may have raised CSF levels</td>
<td>Yes</td>
</tr>
<tr>
<td>Multiple organ failure, sepsis</td>
<td>Neopterin levels correlate with multiple organ failure scores: strong indicator of overwhelming sepsis</td>
<td>Yes</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Similar changes in neopterin as infection with wild virus; neopterin changes precede antibody response and fall as antibodies increase</td>
<td></td>
</tr>
<tr>
<td>HIV</td>
<td>Neopterin concentrations increased in most HIV infected individuals before effects on T cell populations are detectable and long before clinical symptoms; dramatic increase in neopterin in AIDS. Inverse correlation with absolute number of CD4+ T cells</td>
<td>Yes</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>Patients with schizophrenia and endogenous depression present with normal or decreased neopterin levels. Successful treatment associated with normalization of levels</td>
<td>Yes</td>
</tr>
<tr>
<td>Anxiety, stress</td>
<td>Decreasing neopterin levels with increasing anxiety and also during acute stress</td>
<td></td>
</tr>
<tr>
<td>Kawasaki disease</td>
<td>Increased neopterin levels in the acute phase of the disease</td>
<td></td>
</tr>
<tr>
<td>Periodontitis</td>
<td>Increased salivary neopterin</td>
<td>Yes</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
<td>Higher neopterin levels in patients with poor prognosis; more specific for disease progression than C-reactive protein</td>
<td>Yes</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>Elevated neopterin in 70%, but no correlation to tumour stage or specific tumour markers</td>
<td>No</td>
</tr>
<tr>
<td>Alzheimer's disease</td>
<td>Inverse correlation between neopterin levels and mini-mental scores (MMS)</td>
<td>Yes</td>
</tr>
<tr>
<td>Idiopathic cardiomyopathy</td>
<td>No difference in neopterin between cases and controls, but cases with higher neopterin have worse prognosis</td>
<td>Yes</td>
</tr>
</tbody>
</table>

[318, 321, 324, 325, 339, 348-351]
In the Austrian Tyrol, neopterin has been used for screening blood donors since 1986, to detect those with subclinical infectious diseases. In this setting, abnormal is defined as >10nmol/l. Serum concentrations may reach more than 200nmol/l in some diseases [319], with the highest levels being found in endotoxic shock [325]. Elevated neopterin levels in individuals without established diagnosis indicate the presence of (i) acute disease associated with or caused by cellular immune activation or (ii) chronic inflammatory disease [319].

3.11.4 Measurement issues

Neopterin is a small molecule, with no known catabolism in the body and its biological half-life thus depends solely on renal excretion [318, 324]. Renal disease can alter the serum neopterin concentration and if there is a suspicion of renal impairment, neopterin levels should be adjusted for serum creatinine [319, 339]. Neopterin and DHN are both clearly important in physiology, but DHN is typically short-lived and unstable, so that usually only neopterin is measured [318, 324].

Neopterin can be detected in a range of body fluids, including serum, CSF, synovial fluid, pancreatic juice, urine, saliva and ascites fluid [325]. There are three common methods of assaying neopterin: high performance liquid chromatography (HPLC), radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA). HPLC is the method used most commonly to measure urinary neopterin, and can be used with serum samples, but high levels of protein in the serum interfere with the assay and samples must be deproteinised prior to assay [318, 321]. For this reason, for serum samples, RIA or ELISA is preferred.
RIA relies on competitive binding of unlabelled neopterin (in the sample) and radiolabelled neopterin for the binding sites of a polyclonal anti-neopterin antibody. An antigen-antibody complex is formed and the radioactivity of the sample is inversely proportional to the concentration of unlabelled neopterin in the sample [318, 352].

ELISA is a competitive enzyme immunoassay in which sample neopterin competes with a neopterin/enzyme conjugate for binding sites on anti-neopterin antibodies coating the wells of a microtitre plate. The subsequent addition of an enzyme substrate results in a colour change, with the intensity of the colour (measured by the optical density) inversely proportional to the neopterin concentration in the sample [353].

The normal range for serum is $5.3 \pm 2.7 \text{nmol/L}$ in healthy adults [336] with RIA, HPLC and ELISA giving comparable results [345] although ELISA may be slightly superior to RIA in reproducibility of elevated neopterin levels [354]. Commercial kits are available for RIA and ELISA assays of neopterin.

Samples must be protected from direct sunlight, (by enveloping them in tin foil covers), although normal indoor light generally has no effect [319, 355]. Neopterin is otherwise stable [318]. Urine samples can be stored for 2 weeks at 4°C and for at least 2 days at room temperature. Serum samples are stable for at least six months at -20°C.
and can be stored at 2-8°C for up to 24 hours [321, 355]. Frequent thawing and refreezing affects the neopterin levels in samples [318, 355].

**Individual variability**

In a study of healthy blood donors, 8.1% had neopterin concentrations above the accepted 95th percentile figures for their age range (8.7nmol/l), while 2.1% had neopterin concentrations >11.0nmol/l [347]. Among a population of voluntary blood donors, 1.62% had neopterin levels >10nmol/l [356] while 10% of a population of normal volunteers had levels higher than 10nmol/l [357].

A single study has examined variation in neopterin levels over time, showing that while mean population values remained remarkably stable over three months, individual subjects exhibited significant variability, possibly related to subclinical disease [358]. The mean difference in neopterin over three months was 0.15 mmol/l with a range of –3.70 to 4.50.

**Diurnal variation**

Individual neopterin concentration varies diurnally by 10-15% [319] with higher levels at night [318].

3.11.5 **Population distribution**

**Age**

There is significant age variation, with children under 18 years and adults over 75 years having higher neopterin concentrations than adults between these age limits.
There appears to be little age variation in those between 19 and 75 years [318, 321, 325], although Diamondstone reports increasing neopterin with increasing age in adults 20-69 years (20% increase) in a random community sample [359]. Increased neopterin production with age may be due to a higher incidence of diseases such as atherosclerosis or dementia, associated with immune activation, where the pathological process has started but is not yet clinically detectable [336]. Indeed, higher neopterin levels in nonagenarians are associated with reduced life span [347].

**Gender**

There is no apparent gender variation in serum neopterin levels [318, 321, 325, 357].

**Drugs**

Current use of antihistamines is associated with increased levels of neopterin [359].

**3.11.6 Behavioural variation**

**Smoking**

There are contradictory findings on an association between neopterin levels and cigarette smoking, with some researchers reporting no difference [360] while others report lower neopterin levels in smokers compared to non-smokers or ex-smokers [325, 359]. A suppressive effect of tobacco smoke on the human immune system has been hypothesized [347].
Physical activity

Several studies have examined the effect of exercise on neopterin levels, both in elite athletes and in untrained subjects [361-364], showing mildly increased levels with exercise [363, 364], or no change [361, 362]. Smith demonstrated a trend towards depression of neopterin concentration in the very fit [362].

3.11.7 Psychosocial and SES variation

The literature examining the SES variation in serum neopterin and the association with psychosocial factors is summarized in Table 3.6. These findings are covered in greater detail, in the context of my own findings, in the Discussion, Chapter 6.

Table 3.6 Summary of SES and psychosocial variation in serum neopterin.

<table>
<thead>
<tr>
<th></th>
<th>Direction of effect</th>
<th>Findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult SES</td>
<td>No studies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute stress</td>
<td>Inverse association</td>
<td>Perception of stressfulness of the situation alters serum neopterin</td>
<td>[318, 365-367]</td>
</tr>
<tr>
<td>Chronic stress</td>
<td>?direct association</td>
<td>Decreased in post-traumatic stress disorder, but much interindividual variability</td>
<td>[368-373]</td>
</tr>
<tr>
<td>Psychiatric illness</td>
<td>Direct association</td>
<td>Increased levels in anorexia nervosa, depression, seasonal affective disorder. Conflicting results for schizophrenia</td>
<td>[304, 357, 360, 374-378]</td>
</tr>
<tr>
<td>Social support</td>
<td>null</td>
<td>Few studies</td>
<td>[329]</td>
</tr>
</tbody>
</table>

3.11.8 Conclusion

Neopterin is a relatively stable, measurable, and sensitive marker of early immune activation. Urine and serum neopterin levels correlate with the level of immune activation but are not disease specific. While its physiological role and use in disease monitoring are increasingly well understood, little work has been done to determine variation in levels with demographic factors.
3.12 Salivary IgA

3.12.1 Physiology

Saliva

Human saliva is produced from hundreds of minor salivary glands in the tongue, lip and palate in addition to the major salivary glands: the parotid, submandibular and sub lingual glands [379]. Approximately 600-1000ml of saliva is produced daily consisting of water, minerals, electrolytes, buffers, immunoglobulins, growth factors, cytokines, mucins and other glycoproteins, as well as enzymes and enzyme inhibitors [380]. The composition of secretions from different salivary glands differs, as does their response to stimulation by nervous and hormonal mediators.

As well as being involved in maintenance of the oral cavity, digestion of food and lubrication to assist speech and swallowing, saliva is part of the mucosal immune system. This forms a first line of defense against pathogens by providing an acidic environment, mucous to trap, and antibodies (immunoglobulins) to react with, pathogens, allergens and other antigens [379, 380].

Salivary secretion is normally controlled by reflexes, such as chewing and taste, via the autonomic nervous system [381]. The amount and composition of saliva depends on whether the parasympathetic (PNS) or sympathetic nervous system (SNS) is predominantly stimulating secretion. This in turn depends on the nature of the stimulus. Stimulation of the sympathetic nervous system, such as in an acute stress
response, causes a thick, viscous secretion to be formed, whereas stimulation of the parasympathetic nervous system results in a copious watery secretion [382].

**Salivary IgA**

The predominant antibody class found in saliva is of the Immunoglobulin A (IgA) form. While serum IgA exists mainly as a monomer, the form found in saliva is primarily polymeric (pIgA), consisting of two IgA monomers linked by disulphide bonds, with an extra J-chain attached to the end of the heavy chains. In addition, this salivary form of IgA has an attached large carbohydrate-rich glycoprotein derived from the epithelial cell, called secretory component (SC). Thus the salivary form of IgA is termed secretory IgA (sIgA).

**Figure 3.7 Structure of sIgA (adapted from [383])**

![Diagram of sIgA structure](image)

Secretory IgA has a relatively short half-life (3-6 days) and a high synthesis rate (66mg/kg/day) [384]. It is secreted by both major and minor salivary glands under the control of the autonomic nervous system [385]. Most salivary IgA is synthesized and
secreted locally by plasma cells located in the interstitial matrix of salivary glands, then bound to polymeric immunoglobulin receptor (pIgR) on the basolateral membrane of the glandular epithelial cells. The pIgR-IgA complex is internalized and traneytosed across the epithelial cell to the apical surface. Here the pIgR is cleaved from the sIgA and sIgA is released into the saliva [386]. SC represents the cleaved product of pIgR. Figure 3.8 outlines the synthesis and secretion of sIgA.

There are important differences in the concentration of sIgA released from different salivary glands; in particular, saliva from the parotid may have a lower concentration, and minor salivary glands a higher concentration, than saliva from other glands [379, 387]. Preferential collection from a particular salivary gland, or stimulation of salivary flow (which results in greater parotid contribution) will result in spurious variations in the measured concentration of sIgA [379].

Influences on the secretion of saliva are not necessarily synonymous with influences on the secretion of sIgA – differential secretion of saliva and sIgA will have marked effects on the concentration of sIgA bathing mucous membranes and thus resistance to infection. Both the PNS and the SNS are involved in the secretion of sIgA into saliva and the resulting sIgA concentration.

The secretory process is also influenced by hormones including glucocorticoid and thyroid hormones, aldosterone [388] and adrenergic, cholinergic and peptidergic neurotransmitters [389, 390] as well as a number of cytokines including IFN-\(\gamma\), TNF-
α, IL-5 and IL-6 [391]. Nervous and hormonal mediators could affect salivary sIgA levels in saliva at a number of different points: (see Figure 3.8)

1. Stimulation of the plasma cell to increase production, (possibly under parasympathetic and cytokine control);
2. Production of pIgR and uptake of pIgA by the epithelial cell;
3. Transcytosis across the epithelial cell (probably under control of both the SNS and PNS);
4. Release into the duct from the apical/luminal portion of the epithelial cell
5. Salivary flow (under SNS and PNS control)

Both the PNS and the SNS are involved in the secretion of sIgA into saliva and the resulting concentration of sIgA. Evidence from animal and human studies indicates that stimulation of the SNS (as in the acute stress response) results in a markedly increased concentration of sIgA (the net result of a modest increase in sIgA secretion, but decreased salivary flow rate) [385, 392-396]. Stronger effects on concentration than secretory rate have been observed in studies of acute laboratory stress in humans, although increases in secretory rate do occur. The effects of chronic stress on sIgA secretion and the pathways by which these effects are mediated, are less clear.
Monomeric IgA is synthesised in the plasma cell and assembled into polymeric IgA – two IgA monomers connected by a J chain. Secretory components (SCs) are produced in the rough endoplasmic reticulum of the epithelial cell. SC–containing vesicles fuse with the basolateral membrane and this membrane-bound SC interacts with pIgA. This complex is internalized in endoplasmic vesicles, which are transported through the cytoplasm of the epithelial cell, to the apical membrane (trancytosis). The vesicles fuse with the apical membrane of the epithelial cell and sIgA is released into the lumen of the salivary gland, and is available for external secretion.
3.12.2 Diseases associated with salivary IgA

Secretory IgA is part of a mucosal protection system. Individuals with poor salivary flow show an increased incidence of dental caries and other oral infections, and higher levels of sIgA appear to protect against dental caries [397].

A meta analysis of studies examining the association between sIgA concentration and upper respiratory tract illness indicates that reduced levels of sIgA are associated with an increased incidence of upper respiratory tract infections [398]. While a direct cause and effect relationship between low levels of sIgA and clinical illness has not been shown, sIgA levels should be viewed as a risk factor for developing infection. Determinants of the occurrence of infection and subsequent illness are more complex than a simple deficiency of a particular immunoglobulin [399].

3.12.3 Measurement issues

Sample collection

There are many different saliva collection methods. This lack of a standardised method means that results are not necessarily comparable from one study to the next. Variations in methods include:

- Fasting vs non-fasting. Salivary IgA levels from fasting and non-fasting samples are not comparable, with fasting samples having a much wider normal range than non-fasting samples. However, some studies fail to control for fasting status and may compare fasting and non-fasting samples [396, 400]. Most studies collect saliva from non-fasting subjects, or fail to specify fasting status.
• Stimulated vs unstimulated. Chewing stimulates saliva flow and decreases sIgA concentration [401, 402]. However, sIgA secretory rate is higher during chewing than at rest [401, 402], i.e. both output of sIgA and saliva flow are increased by chewing, but the latter undergoes a larger increase than the former, resulting in a net decrease in sIgA concentration, with chewing. While some authors report higher correlations [401], others report greater intra-individual variability, using stimulated samples [379]. Most studies collect and measure saliva without stimulation.

• Timed collection. Recent studies collect saliva for a timed period in order to calculate the saliva flow rate and thus sIgA secretory rate (see below for detail). Collection time varies from 2 minutes to 5 minutes, depending on the method of collection, e.g. two minutes for absorption onto a Salivette [403], 4-5 minutes for dribbling and spitting collections [381, 400].

• Collection method. Variations include spitting [381, 404], dribbling into a cup [400], suction using a suction pump [405], absorption of saliva using a Salivette [403], or the use of other cotton absorbent material [406]. Collection methods using Salivettes or other cotton absorbent material may be associated with unsystematic error in the sIgA measurement, with significantly lower levels of IgA recorded [406, 407]. Comparison of saliva collection by spitting or suction indicates that IgA concentrations collected by these methods are similar [407].

Anything that causes oral trauma (e.g. teeth cleaning) may spuriously increase the levels of salivary IgA due to contamination of the samples with blood [406].
Sample storage

After collection, samples should be placed immediately on ice to prevent degradation of sIgA by salivary proteolytic enzymes [379] and stored at -20°C [381, 403, 408]. Long term storage does not cause degradation of sIgA [379].

Method of analysis

Salivary IgA can be analysed using either an enzyme linked immunosorbent assay (ELISA) or radial immunodiffusion (RID) assay. The RID assay was developed to measure serum IgA, and uses a correction factor to adjust results to saliva levels. There is some dispute as to the size of this correction factor and thus some authors recommend ELISA rather than RID [409]. Comparison of the two tests suggests that ELISA methods may be more sensitive, less variable and cheaper than RID [410].

(Note that salivary IgA assays measure total salivary IgA, i.e. secretory IgA as well as IgA without the secretory component, for example due to blood contamination of the sample).

Concentration or secretory rate?

Researchers continue to debate the relative merits of measuring IgA concentration, calculating IgA secretory rate (from timed saliva flow rate and concentration of IgA) or measuring the ratio of salivary IgA to salivary protein. While there are good theoretical reasons to measure secretory rate, changes in concentration appear to be more sensitive to psychological stressors [395, 396, 411, 412], and to relaxation techniques [411].
The concentration of IgA in saliva (mg/ml) is a function of both secretory rate (the rate at which IgA is transferred from the basal to the apical membrane, released into the lumen of the salivary duct and thence into the saliva) and the salivary flow rate. Secretory rate is expressed as mg/min and salivary flow rate as ml/min. Thus,

\[
\text{Concentration (mg/ml)} = \frac{\text{Secretory rate (mg/min)}}{\text{Salivary flow rate (ml/min)}}
\]

Which parameter(s) to measure depends on the research question. If we are interested in the mechanism by which stressors (exercise etc) affect salivary IgA, then secretory rate (a measure of how fast IgA is secreted into saliva) is of interest. The research question might be: does stress work via increases in secretory rate or simply via changes in salivary flow rate, or a combination of these? If, on the other hand, we are interested in salivary IgA as a marker of stress, then the correlation in experimental studies appears to be higher and more consistent with IgA concentration (reflecting stress-related alterations in both salivary flow rate and secretory rate). If we are interested in the way in which stressors may affect health and the incidence of upper respiratory infections then we are probably most interested in the absolute amount of salivary IgA bathing the mucous membranes [384, 413]. This is a combination of sIgA concentration and salivary volume (as distinct from flow rate).

The ratio of sIgA to salivary total protein concentration has been used to control for salivary flow rate (commensurate increases in sIgA and total protein should be due to increases in salivary flow rate rather than sIgA secretion rate). However, under some
circumstances, e.g. exercise, there is an increase in secretion rate of salivary proteins and so a decrease in the ratio of sIgA to protein, without any change in sIgA output or salivary flow rate [414].

In summary, the choice of measure should be tailored to the question being investigated and will determine the collection method (since timed saliva flow is not appropriate with certain collection methods).

**Temporal reliability**

Mean population values of salivary IgA are very stable over 3 months, but there is considerable intraindividual variability [358]. Estimates of test-retest correlation for concentration of salivary IgA range from (Pearson correlation coefficient) 0.43 over 1-2 weeks [381], to 0.66 (after a two year interval, [401]). Test-retest correlation coefficients for secretory rate tend to be lower, ranging from 0.16 (over 4 weeks), [415] to 0.58 (over two years) [401].

**Concurrent disease**

Gleeson (1995) observed elevations of salivary IgA in 90% of episodes of acute respiratory illness in children aged between 6 months and 5 years. Such elevations commenced, on average, two days after the appearance of symptoms (Day 2), reached a peak on Day 5 (range 2-6 days) then returned to pre-infection levels by Day 10 [416].
Season

While Deinzer et al [417] raise the possibility that seasonal effects may have explained their findings in relation to the effects of academic stress on salivary IgA concentration, there are few studies that specifically address this question. Park and Tokura (1994) found that salivary IgA concentration was significantly higher in those wearing half-sleeve shirts and knee-length trousers than those in long sleeve shirts and full length trousers, presumably related to temperature effects (cited in [418]). In a small group of women, Li et al found a significant increase in IgA concentration from autumn to winter in those who wore knee length skirts, but not in those who wore trousers. It was postulated that this was an effect of SNS stimulation by colder temperatures [418]. The possibility of seasonal variation requires further investigation.

Diurnal rhythm

Salivary IgA concentration peaks on awakening, falls steeply over the next four hours, then levels flatten out and remain relatively stable over the day [389]. This diurnal rhythm also applies to secretion rate of sIgA. The morning peak is dependent on waking, rather than time of day, while the rate of decline of sIgA after waking is positively correlated with the simultaneous rise in salivary cortisol [384, 389]. This diurnal pattern is present in 20-30 year olds, but absent in 60-80 year olds [384]. Collection of saliva should be standardised for time of day, or have time of collection included as a variable in the analysis [419].
**Hydration**

Dehydration is associated with decreased saliva flow, and the mild dehydration commonly seen after exercise in non-elite athletes may explain the slightly increased levels of sIgA seen in this situation [379]. (Note that decreased saliva flow with no change in secretion of IgA will result in an increased concentration of sIgA.).

### 3.12.4 Population distribution

**Age**

Salivary IgA concentration tends to be lower in children less than 7 years of age than in older children or adults [411, 420], with a significant positive linear relationship between age and concentration of salivary IgA in children [420]. Healthy elderly persons (>70 years) have higher concentrations of salivary IgA compared to adults (25-50) years (median 205.4 mg/L vs. 113.4mg/L, p<0.001) [421], due to lower saliva flow rate but relative preservation of IgA secretion rate [384]. However, the absolute amount of IgA bathing mucosal surfaces may be decreased, with a resultant decrease in mucosal protection and increased susceptibility to disease.

**Gender**

There is no apparent gender effect on salivary IgA levels [388, 396, 422].

### 3.12.5 Behavioural variation

**Smoking**

Cigarette smokers have lower levels of salivary IgA [416].
Physical activity

Current research suggests that training at an intense level for many years may cause chronic suppression of salivary immunoglobulin levels [379, 419], and this may be linked to increased respiratory illness in these athletes [419]. Exercise in non-elite athletes is associated with no change or slightly increased concentration of salivary IgA and this is probably a result of mild dehydration during exercise, as already noted [419].

Diet

Vitamin A deficiency may be associated with a diminished salivary IgA response to infection, through impairment of the T_H2 mediated immune response [423, 424]

3.12.6 Psychosocial and SES variation

The literature examining psychosocial and SES variation of salivary IgA is summarized in Table 3.7. These findings are covered in greater detail, in the context of my own findings, in the Discussion, Chapter 6.

3.12.7 Conclusion

Activation of the acute stress response and thus the sympathetic nervous system is associated with acute increases in both concentration and secretory rate of salivary IgA. However, the effects of chronic stress are less clear. There is considerable debate around the most appropriate collection method and whether to measure secretion rate or concentration and there are a number of issues in the collection and analysis of samples.
Table 3.7 Summary of SES and psychosocial variation in salivary IgA.

<table>
<thead>
<tr>
<th>SES and Psychosocial Variation</th>
<th>Direction of effect</th>
<th>Effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult SES</td>
<td>Direct association</td>
<td>With secretory rate, explained by smoking</td>
<td>[425]</td>
</tr>
<tr>
<td>Acute stress</td>
<td>Direct association</td>
<td>Inconsistent results depending on IgA parameter measured, but generally a direct association.</td>
<td>[392-394], [426-428]</td>
</tr>
<tr>
<td>Subacute stress</td>
<td>Inverse association</td>
<td>Examination stress; generally decrease in the lead up to exams. One study showed a direct association, one a null association.</td>
<td>[429], [415], [409], [417], [430]</td>
</tr>
<tr>
<td>Chronic stress</td>
<td>Inverse association</td>
<td>Daily hassles, life events, occupational strain, isolation, natural disasters. Generally consistent; one direct association but no control for personality, dose response relationship. Response may be modified by personality.</td>
<td>[381, 431-436]</td>
</tr>
<tr>
<td>Relaxation, Positive mood</td>
<td>Consistent increase in sIgA concentration and secretory rate with activities promoting relaxation.</td>
<td>[437], [403, 436, 438, 439]</td>
<td></td>
</tr>
<tr>
<td>Social support</td>
<td>Direct association</td>
<td>Higher sIgA in those with high social support in the pre-exam period.</td>
<td>[415]</td>
</tr>
</tbody>
</table>

There are limited data on the temporal reliability of salivary IgA concentration. Yet saliva provides a non-invasive method of examining the effects of stress on the humoral immune system. Despite limitations in our knowledge of the physiological effects of chronic stress on the mucosal immune system, salivary IgA is included as a biological marker in this study, both to complement the other, blood-based markers, and as a measure of the humoral immune system.
The five biological markers, outlined above, have been selected to test the following hypothesized pathway from SES to ill health:

\[ \text{SES} \rightarrow \text{psychosocial stressors and psychosocial assets} \rightarrow \text{biological markers} \rightarrow \text{disease} \]

The preceding discussion has presented evidence of the links between the physiological stress response, and the biological markers, as well as the evidence for links between the biological markers and disease.

The Biomarkers Study collected data to examine the links between SES and psychosocial stressors and assets, as well as between SES and the biological markers. Statistical analysis of these data provides information on the mediating role of psychosocial stressors and assets in the relationship between SES and the biological markers.
Chapter 4.

Methods

4.1 Introduction

The Biomarkers Study consists of two quite separate periods of data collection – a small pilot study in 2000 and the larger main study in 2001. In this chapter, I initially describe the two studies and then the general methodology that is common to both.

This is a cross-sectional study using a sub-sample of the 40-44 year old cohort of the Personality and Total Health Study (PATH Study). Data collection included that obtained from questionnaires, body measurements and biological samples. (Copies of the questionnaire and a detailed description of the methods used in analysis of the biological markers not routinely measured are contained in Appendices B, C and D).

I begin the chapter by describing the PATH study, and then discuss the sampling strategy for the pilot study and the 2001 study. I describe the mechanics of data collection and then summarize the psychosocial, socioeconomic, behavioural and health variables measured in the questionnaire, including details of the instrument used to measure each one and the rationale for this choice. I then build on the previous discussion of the biological markers, by describing sample collection and marker assay. Finally I discuss data management, issues of bias and representativeness and outline the analysis that was undertaken.
4.2 The Personality and Total Health Study (PATH study)

The PATH Study is being conducted in Canberra, by the Centre for Mental Health Research at the Australian National University. It is a twenty-year prospective cohort study. In the initial wave, individuals were randomly selected from the Australian Electoral Roll to form three cohorts at different age groups - 20-24 years, 40-44 years and 60-64 years. In 1999, 2,500 individuals in the 20-24 year age group were interviewed and in 2000 and 2001 this was repeated for the 40-44 year age group and the 60-64 year age group respectively.

The research aims to examine the mental and physical health of adults and to investigate how lifestyle, social and genetic factors might influence this. Each cohort is to be re-interviewed every four years.

The Centre for Mental Health Research kindly allowed the Biomarkers Study to be added onto the PATH project for the 40-44 year age cohort of 2000. The questions asked of the participants in the PATH study allowed the selection of a sub-sample of healthy adults whose socioeconomic status as assessed by occupation and education was known. In the Biomarkers Study this sub-sample was studied in more detail with further questions on socioeconomic status and various psychosocial factors and with the collection of biological data from blood and saliva.

4.3 Recruitment of participants

At the end of each PATH study interview, participants were given an information sheet about the Biomarkers project. After reading this, each participant indicated
whether or not they were interested in being contacted regarding participation in this project and this was recorded.

From the data obtained at the PATH study interview, occupational information was coded using the Australian Standard Classification of Occupations 2nd ed (ASCO 2nd ed). On the basis of these codes, occupational status codes were assigned using the ANU3_2 coding system and the participant ID numbers sorted by occupational status (details of the ASCO 2nd ed. Coding and the ANU3_2 occupational status scale are presented in section 4.6.1). Difficulties in assigning ASCO codes were resolved by the Australian Bureau of Statistics (ABS).

4.3.1 Pilot study

The occupations of the first 500 participants in the PATH study who had indicated that they might be willing to participate in the Biomarkers of Social Disadvantage Study were coded as outlined above.

The PATH study measured self-rated health, limitation of daily activities due to health and participant status for a number of specific health problems, including hypertension, heart disease, asthma, cancer and diabetes. Only those PATH participants with self-rated health described as “good”, “very good” or “excellent”, who had no, or minimal limitation of activities due to health, and suffering from none of the specified diseases were included in the study population. Individuals who were no longer resident in the ACT were also ineligible for inclusion in the Biomarkers Study.
Within this study population, participants were ordered by occupational status and the pilot study sample was chosen to include the top 15 participants of each gender, and the bottom 15 of each gender. Different measures of SES, such as occupational level (ASCO coded), occupational status and education are highly correlated. To confirm that these two groups did indeed form two distinct SES groups, occupational level and education of the chosen study sample were examined, but final selection was on the basis of occupational status. In the lower SES group women had an occupational status <29 and men <34, while in the upper SES group, women had an occupational status of >65 and men >75.

Figure 4.1 outlines the pilot study sample selection and data collection.

These 60 people were contacted by mail to indicate that they had been chosen for this study and would be contacted by telephone soon. The questionnaire was included in this mail out and people were asked to fill this in and bring it to the interview. Once contacted by telephone, participants were asked if they were still willing to participate and if so, an interview time was arranged to be approximately two hours post-prandial (to allow saliva samples to be taken). Participants were requested to phone and change the appointment if they became unwell, e.g. upper respiratory infection, as this may alter the salivary IgA concentration. If this occurred the interview was rescheduled to be at least two weeks after the person was feeling well again. Any persons who were no longer willing to participate were dropped from the sample and the next eligible participant on the basis of status was contacted.
Figure 4.1 Pilot Study outline

First 500 PATH-yes

Ineligible if:
- Self-rated health less than “good”
- Suffer from specific diseases
- Moved out of area

Total study population
265 people

- Occupational coding
- Assign occupational status
- Order by occupational status

Ordered list of study participants
(by occupational status), separated
by gender

Two groups at extremes of
occupational status, gender
balanced (total 60 people)

Refuse participation?
Mail out and contact next
person on status ranking, gender-matched

- Mail out information and
questionnaire
- Contact and interview time
(two hour post-prandial)

Consent
Questionnaire
Body measurements
Blood and saliva tests

Feedback

Three months later

Feedback

Blood and saliva tests
At the interview, each participant signed a consent form and was invited to ask any questions relating to the study. The completed questionnaire was collected, or if not completed, a reply paid envelope was given to facilitate return of the questionnaire. The pilot study questionnaire requested information on personal income, household income, childhood socioeconomic status on the basis of education and occupation of parents at participant age 12, and indicators of household wealth, i.e. car and house ownership. It contained measures of self-esteem, coping style, social capital, economic strain, social support, and optimism.

Height and weight, waist and hip circumference were measured and a saliva sample taken. A full explanation was given to each participant as to what we were looking for in the blood test and an explanation about why the sample was a fasting sample and what this involved. The form for the blood test was given to the participant, with a request to complete this at a Capital Pathology collecting centre, as soon as possible. Any problems with completing the blood test were discussed, e.g. inability to attend a collecting centre during the opening hours, and solutions sought.

Participants elected on the questionnaire and were again asked at interview how they wanted the blood results dealt with, and were encouraged to be informed about the results. Results of blood tests were phoned through to participants and then a copy mailed to general practitioners (GPs) if this was requested.

Ten weeks after the initial contact was made, the participants were contacted again to invite them to provide a further saliva sample and undergo another blood test.
Participants were encouraged to have the second blood test as close to three months from the original blood test as possible. Results of blood tests were reported back to participants, as above.

### 4.3.2 2001 study

Following completion of the PATH 40-44 years wave in early 2001, occupations of all participants were coded to occupation and then to occupational status (as above). All occupational codes were checked and rechecked to ensure they were consistent and accurately fitted the job title and description.

Of the total PATH sample, those who did not want to participate were dropped. All those whose self-rated health on the PATH Study questionnaire was poor or fair were excluded as were all those who stated that they had diabetes, heart disease, hypertension or cancer, or stated on the SF-12 that they were limited a lot by their health. Only those who stated their race as “Caucasian” and who remained resident in the Canberra/Queanbeyan region were retained in the study population.

The remaining possible participants were sorted by occupational status and gender, and then a histogram of occupational status was constructed for each gender. There was a fairly clear delineation into four groups for both men and women, with cut-offs at similar levels of occupational status for both genders.
The PATH Study identification (ID) numbers in each group were ordered from lowest to highest in an Excel spreadsheet, with no separation by gender. Using a list of random numbers, 100 possible participants were chosen from each group. The first 75 in each group formed the participants of the 2001 study, while the remainder formed an ordered list of possible replacements for any of the first 75 who for any reason could no longer participate in the 2001 study.

The four groups based on occupational status were deliberately not sorted by gender prior to random selection of participants, so that the selected individuals would reflect the gender makeup of the underlying population within the same range of occupational status. While the total sample is clearly not representative of the
Canberra population (since it has been selected to give equal numbers across the SES spectrum), each status group should be representative of, and the results generalisable to, the corresponding population group.

Because a significant period elapsed between the PATH study interview and the 2001 study (up to about 20 months) it was necessary to update information on some of the variables that may have changed in the interim, e.g. life events. The 2001 study questionnaire thus contained these scales from the PATH study, the questions from the pilot questionnaire and additional items on social capital. However, the resultant questionnaire was quite large and it was thus split into two questionnaires. The first was similar to the pilot questionnaire, asking essential information on variables not covered in the PATH study and was sent out with the introductory letter in the same way as in the pilot study. A second questionnaire was then given out at interview, with the explanation that it was an update of some of the PATH questions, with a reply-paid envelope to encourage its return.

Figure 4.3 outlines the 2001 study sample selection and data collection.

Contact was made by mail out and then telephone call. During the telephone call the individual was first asked if they were still interested in participating. If this was affirmative, they were asked to indicate their self-rated health (poor, fair, good, very good or excellent) and then whether they had developed any of heart disease, cancer, diabetes, high blood pressure or other major illness. Provided self-rated health was good or better and the person was healthy and still resident in the study area, an
interview time was made. None of those contacted had poor or fair health, but a number had developed major illnesses and were excluded from the study.

The interview took place in the same way as in the pilot study. Participants were interviewed two hours post-prandially, and a saliva sample and body measurements were taken. In this part of the study, a blood pressure reading was taken. The blood tests were explained and a blood form given to have fasting bloods collected at an ACT Pathology collecting centre.

All participants were notified of their blood results by telephone and letter and were sent an additional copy of the blood results for their general practitioner.

Each time a participant was found to be ineligible to participate, or unwilling to participate, the next ID number on the list of random ID numbers for the same SES group, was contacted to invite participation.

At all times, the interviewer was blind to the SES group to which the participant belonged. The grouping was recorded only on an Excel spreadsheet where the PATH ID number identified possible participants. The ID numbers randomly chosen were given to the PATH study data manager, who then assigned a Biomarkers ID number and provided contact details. The group to which an individual belonged was only traced when a replacement needed to be made.
Figure 4.3 2001 study outline

40-44 year PATH study sample
2530 people

Interested in participating in Biomarkers study

40-44 year PATH-yes sample
2042 people

Ineligible if:
- Self-rated health less than “good”
- Suffer from specific diseases
- Moved out of area

2001 Study population
1248 people

- Occupational coding
- Order by occupational status
- Separate into four groups based on occupational status
- Randomly select 100 people from each status group

First (randomly selected)
75 people from each group form study sample

Refuse participation?
- Mail out and contact next randomly selected person from same status group

Consent
Body measurements
Blood and saliva tests
Second questionnaire to mail back

Feedback results to patient and GP

Data entry, manipulation and analysis
4.4 Ethics

Prior to commencement of the Biomarkers Study in 2000, ethics approval was obtained from the Human Research Ethics Committee of the Australian National University. The research was carried out in accordance with the ethical guidelines set out by the National Health and Medical Research Council in the National Statement on Ethical Conduct in Research Involving Humans.

The following sections detail the study measures used in the pilot and the 2001 studies.

4.5 Psychosocial factors

Hemingway and Marmot define a psychosocial factor as “a measurement that potentially relates psychological phenomena to the social environment and to pathophysiological changes” [116]. The following psychosocial factors were examined in this study.

4.5.1 Social support

No single measure is recognized as being the “gold standard” for the measurement of social support in health research. One can measure perceived social support of an instrumental (support with money, accommodation and other concrete items) and/or emotional type [440, 441], or actual number of individuals in the social network (that therefore should be available to provide support) [442]. Few measures incorporate the notion that social interactions can be negative and have detrimental health effects.
The PATH study uses the social support scale developed by Schuster et al [443] to measure both positive and negative aspects of social support. The authors postulate that social support derives from several different sources (spouse, relatives, and friends) and may entail both positive and negative interactions, which may moderate the health effects of stressor exposure.

The scale measures positive and negative interactions separately for friends, family and partner. Schuster reports alpha reliabilities of greater than 0.6 and in the 2001 study Cronbach’s alpha coefficients for these six subscales were between 0.68 (negative interaction with friends) and 0.91 (positive interaction with partner). As per the original scale, all indices were scaled so that the theoretically lowest scores were coded to 0 and the theoretically highest scores were coded 1.

This scale has been used to examine the effects of social support on mental health and well-being [443] and has clearly shown that negative social interactions (as measured by the scale) are associated with higher levels, and supportive interactions with lower levels, of psychological distress.

In addition to the Schuster scale, an additional question asking about the number of close friends formed the final element of the assessment of social support/networks.

4.5.2 Childhood adversity and parenting

Questions on childhood adversity and parenting used in the PATH study were derived from the Parental Bonding instrument [444] and the National Comorbidity Survey.
Responses to these questions were taken from the data collected as part of the PATH study, i.e. they were not re-measured in the Biomarkers Study.

The questions used in the PATH study represent two items from the Parental Bonding Instrument: “How affectionate was your mother (father) towards you?” and several questions that are a summary of questions from the NCS. The NCS contained twenty-six childhood adversities consisting of:

- Five interpersonal loss events – death of mother, death of father, parental separation or divorce, parental absence of 6 months or more other than due to separation or divorce, respondent absence of 6 months or longer.
- Eight interpersonal traumas – maternal and paternal verbal aggression, isolated rape, repeated rape, sexual molestation, being mugged or held captive and being seriously physically attacked.
- Eight measures of parental psychopathology – major depression in mother or father, generalized anxiety disorder, alcohol or drug abuse and antisocial personality disorder in either parent when the respondent was a child.
- Five miscellaneous adversities – being in a life-threatening accident, being in a natural or man-made disaster, learning about a traumatic experience that occurred to a close loved one or witnessing a traumatic event.

Of these, the PATH questions cover divorce or permanent separation of parents, parental psychopathology and drug abuse and sexual, verbal and physical abuse. There are also three items that rate respondent satisfaction with their childhood and upbringing.
Exposure to childhood adversity is commonly reported in those who suffer psychiatric disorders as adults [445] and there is mounting evidence that the childhood environment is important in setting the stage for later resilience or vulnerability to stressful life events [446].

4.5.3 Social capital

In the pilot study, personal social capital was assessed by questions from the US General Social Surveys, looking at civic trust, (“Generally speaking would you say that most people can be trusted?” or “You can’t be too careful in dealing with people.”) and perceptions of reciprocity, (“Would you say that most of the time people try to be helpful, or are they mostly looking out for themselves?”) [447]. A sense of belonging to the community was assessed by asking whether there was any membership of community groups and if so, how many. Using these very simple questions, Kawachi et al [447] have shown high correlation at a group level between low levels of social trust and fair or poor health.

In the 2001 study, the social capital measure included the above measures as well as questions regarding the participant’s sense of belonging in the community and social trust.

The belonging scale is part of Cohen’s Interpersonal Support Evaluation List (ISEL) that is widely used in health research to measure social support [448]. It consists of ten statements requiring a true/false response, such as “No one I know would throw a
birthday party for me.” Half of the statements are positively worded, half negatively worded. Responses are scored out of a possible total of ten, with one point for each positively worded item marked as true and one for each negatively worded item marked as false. Cohen has demonstrated adequate test/retest reliability (0.82 over four weeks) and good internal reliability (alpha coefficient = 0.75-0.78) [448]. In the 2001 study, the alpha coefficient for this scale was 0.81.

The trust scale is a four-item scale derived from the Organizational Trust Inventory (OTI) developed by Cummings and Bromiley, 1996 [449]. Participants are asked to agree or disagree with statements such as, “Most people don’t mislead others”, on a seven point Likert scale from “definitely disagree” to “definitely agree”. Cronbach’s alpha in the 2001 study was 0.87.

### 4.5.4 Optimism/Pessimism

The Life Orientation Test (LOT) developed by Scheier (1985), is commonly used to evaluate an individual’s sense of optimism [450]. It indicates optimism/pessimism as a relatively pure trait, although there is some overlap with coping, self-esteem, self-mastery and neuroticism. In 1994, Scheier and Carver revised the LOT to narrow the original instrument down to a more pure measure of the construct of optimism [451]. The revised LOT (LOT-R) is used in the Biomarkers Study.

The LOT-R is a measure of dispositional rather than situational optimism. It is a ten-item scale with four filler items, three positively worded items (“In uncertain times, I usually expect the best”) and three negatively worded items (“I hardly ever expect
things to go my way”). It is scored on a 5-point scale from “strongly agree” to “strongly disagree”. Negatively worded items can be reverse-coded and summed with the positively worded items, to give a single score, or the positively and negatively worded items can be scored separately. There is some evidence that the separately scored negatively worded items have a stronger relationship with SES and health outcomes [452].

Internal consistency is satisfactory, with a reported Cronbach’s alpha of 0.78 and test-retest reliability of 0.79 after 28 months [451]; and Cronbach’s alpha for the 2001 study of 0.83.

4.5.5 Stressful Life Events

The PATH study uses Brugha and Cragg’s List of Threatening Experiences (LTE) [453] to examine the number of recent stressful life events. This is a 12-item questionnaire, which asks whether or not a series of potentially stressful events have happened to the individual over the last six months. Items include: death of a close family friend or relative, a crisis or disappointment at work or the end of a steady relationship. In addition, two questions ask about stressful life events affecting the participant’s partner.

Test-retest reliability has been demonstrated of >0.75 (Cohen’s kappa) and there is good correlation (Cohen’s kappa >0.63) between the LTE questionnaire and a contextually rated long-term threat rating, from interview [453]. Life events can be simply scored, i.e. there were 6 serious life events in the last six months, or a
weighting can be applied to the various items, i.e. death in the family is more stressful than loss of something valuable. In the Biomarkers Study the number of life events was summed without weighting. To adjust for the absence of a partner (and thus a lower possible total life events score) the total derived by summing, was divided by the number of items, to give each individual a score out of 1.

4.5.6 Affect – PANAS scale

Affect can be examined as a state (“how you are feeling now”) or as a trait (“how you usually feel”). For the purposes of this study, the latter is most important and is considered to be a stable personality characteristic. In the PATH study, the positive and negative affect scale (PANAS) [454] is used to measure affect, and this was repeated in the 2001 study. Positive and negative affect are not two ends of the same spectrum. The person high in PA (positive affect) is energetic, enthusiastic, alert and active, while the person low in PA is lethargic, bored and sad. The person high in negative affect (NA) is angry, hostile, fearful and contemptuous of others, while the person low in NA is calm and serene. In both the PATH and the 2001 studies the PANAS has added items to more fully assess trait hostility (from the PANAS-X scale, [455]). The scales of the PANAS have been demonstrated to have good test/retest reliability and high internal consistency. In the 2001 study, Cronbach’s alpha coefficients for each scale were: PA = 0.91; NA = 0.89; Hostility = 0.80.

The scales consist of a number of words that describe different feelings or emotions. Respondents are asked to rate (on a scale of 1-5, where 1 = “very slightly or not at all” and 5 = “extremely”) to what extent they have been feeling this way over the last
month, e.g. disgusted, alert, proud. The version of the PANAS-X used has ten items to assess PA, ten to assess NA and six items to more fully assess hostility (four extra items and two items, hostile and irritable, that are part of both the NA scale and the hostility scale). For each subscale, the ratings are added to obtain a score for PA, NA and hostility.

4.5.7 Affect - Behavioural inhibition/activation

The behavioural inhibition system (BIS) is related strongly to negative affect as measured by the PANAS, while the behavioural activation system (BAS) relates strongly to positive affect. However, the BIS and BAS scales are thought to reflect the sensitivity of the individual to anxiety (BIS) or impulsivity (BAS) given a particular situation, whereas the PANAS measures emotions actually experienced. Thus a person may be vulnerable to anxiety in certain situations, but have learned behaviours to decrease that anxiety and thus experience less anxiety. The BIS and BAS scales are thought to tap more inherent elements of personality, whereas the PANAS looks at the net effect of this inherent personality plus some learned coping behaviours [456].

The BIS/BAS consists of a number of statements in which respondents rate the level of agreement with a statement, on a scale of 1 = “very false for me” to 4 = “very true for me”. The BIS/BAS scale is made up of four sub-scales: the BIS, the BAS Reward Responsiveness, the BAS drive and the BAS Fun Seeking. The BIS scale examines sensitivity to the anticipation of punishment, with such questions as “I worry about making mistakes”. The BAS Reward Responsiveness scale examines the extent of
positive reactions to the anticipation of a reward, e.g.” It would excite me to win a contest”. The BAS Drive scale looks at persistence in the pursuit of goals, with items such as “When I go after something I use a “no holds barred” approach”. The BAS Fun Seeking scale reflects the impulsive seeking of fun and excitement, e.g. “I crave excitement and new sensations”.

Carver and White demonstrated reasonable test-retest correlations (0.66 for BIS, 0.66 for Drive, 0.59 for Reward Responsiveness, and 0.69 for Fun Seeking) after 8 weeks [457]. Internal reliability is high, with reported Cronbach’s alpha of 0.74 BIS, 0.73 Reward Responsiveness, 0.76 Drive and 0.66 Fun Seeking in the original study and the same or higher in Jorm’s application of the BIS/BAS to an Australian community sample [456]. In the 2001 study Cronbach’s alpha coefficients were high for all scales: BIS = 0.84; Drive = 0.82; Reward Responsiveness = 0.71; Fun Seeking = 0.73.

4.5.8 Neuroticism, Psychoticism, Extroversion

These personality traits are measured in the PATH study and subsequently in the 2001 study using the short version of the Eysenck Personality Questionnaire – Revised scale [458]. Requiring “Yes/No” answers, the EPQ-R contains simple questions such as, “Are your feelings easily hurt?”, “Do you enjoy meeting new people?”, and “Do good manners and cleanliness matter much to you?” Groups of questions load onto each of the above facets of personality - neuroticism, extroversion and psychoticism.
There are 12 items for each of neuroticism, extraversion, and psychoticism, each scored 0 or 1. Items are then totalled out of a maximum of 12 for each subscale. Where there are missing values, within a subscale, provided there are ten or more non-missing values, the mean of these is taken and multiplied by twelve to obtain a final score.

The reported alpha reliabilities for the extroversion and neuroticism scales are high (>0.8) while that of the psychoticism scale is not as high, but satisfactory (>0.6) Test-retest reliability for the scale is >0.7 [458]. In the 2001 study, the alpha coefficients were similarly high for the extroversion and neuroticism scales (0.88 and 0.81 respectively), but rather low for the psychoticism scale (0.46).

4.5.9 Perceived Stress

“Stress” is as difficult to measure as it is to define. An explicit definition makes measurement possible, but still reflects only one perception of stress. In the pilot study stress is measured by assessment of life events from the PATH study as well as questions on economic strain. Following suggestions by participants in the pilot study, some of the items measuring economic strain were dropped in favour of questions simply asking people how they assessed their stress levels and what the causes of this stress might be.

In addition, a ten-item scale was introduced in the 2001 study to examine perceived stress more formally. The perceived stress scale (PSS) developed by Cohen et al examines the participant’s appraisal of how stressful particular situations have been,
over the last month [459]. It is a global measure of perceived stress, looking at difficulties in general, rather than specific events such as a life events scale examines.

The PSS has been used in a number of studies examining links between stress and both physical and mental health [460-464].

Participants answer questions such as “In the last month, how often have you felt that things were going your way?” on a 5-point scale from 0 = “Never” to 4 = “very often”. A total score is achieved by reverse coding the positively worded items and then summing across all ten items, i.e. a maximum score of 40 indicating a high level of perceived stress. Cronbach’s alpha is reported as 0.87 for the 14-item version of the PSS [463] and 0.91 for the 10-item version [461]. In the 2001 study, Cronbach’s alpha for this scale was 0.86.

4.5.10 Economic strain

In the pilot study the economic strain variable had three components, based on Pearlin’s model of economic stress [465]. These were:

- Economic strain, a 9-item scale asking questions about whether the participant felt he/she could afford to buy several essential and non-essential items.
- Economic positive comparisons (EPC), a six-item scale that asks the participant to rate their economic circumstances against the perceived economic circumstances of others in the community. Thus a person, of any income, who feels that friends, relatives and neighbours are better off, is likely to feel some economic stress.
• Devaluation of the importance of money model (DIMM). This is a 3-item scale, which seeks to see how participants might reframe how unimportant money is to them, to cope with perceived lack compared to peers.

Comments by participants in the pilot study suggested that the questionnaire was too heavily based on economic stress and several participants were unwilling to make comparisons of their economic circumstances with others (in the EPC) or had difficulties answering the questions on the DIMM. For the 2001 study, therefore, the latter two scales were dropped. The economic strain scale was retained (alpha coefficient in the 2001 study = 0.80) and a new three-item deprivation scale was added – “Do you go to the dentist for routine dental checkups?”, “Does your partner go to the dentist for routine dental checkups?” and “Do you have household contents insurance?”

4.5.11 Coping

Coping strategies are assessed with the Brief COPE [466], an abbreviated version of Carver and Sheier’s COPE scale [466]. Both COPE scales have been widely used in health research.

The original COPE scale consisted of 60 questions that loaded onto 15 coping strategies, e.g. active coping, mental disengagement, denial, substance use, use of emotional support, use of instrumental support, behavioural disengagement etc. The

* Contributed by Helen Berry, PhD scholar, Centre for Mental Health Research
Brief COPE is shortened to 28 questions loading onto 14 slightly different types of coping strategies.

In the both the pilot and the 2001 studies, dispositional (rather than situational) coping is assessed by asking participants to “think about what you usually do when you are under a lot of stress”, rather than asking how the person coped in response to a specific event or over a specific time period.

The Brief COPE was used in both parts of the Biomarkers study to measure coping styles in a concise, but theoretically rigorous way that would not overload participants who also had to respond to a number of other questions. The scale consists of 28 items asking the participant to rate, on a four point scale, how much they would use this action in response to a stressful event, from, “I don’t usually do this at all” (1), to “I usually do this a lot” (4). Each of the fourteen subscales consists of two items, to provide a total score out of eight for each coping strategy. The alpha coefficients in the 2001 study, for the subscales measuring use of humour, acceptance and self-distraction were relatively low (<0.40); the subscales measuring denial, behavioural disengagement, and venting had modest alpha coefficients (0.51-0.65); the remaining subscales had high alpha coefficients (>0.74).

4.5.12 Work Stress

Occupational stress was assessed using Karasek’s job control/demand model [467] with the questionnaire used by Bosma et al [75] in the Whitehall studies. Of fifteen job control items, nine cover decision authority and six cover skill discretion. The
subscales are equally weighted and can be summed to give a job control scale which has been reported to have good internal consistency (Cronbach’s alpha = 0.84) [70]. In the 2001 study, Cronbach’s alpha for the skill discretion and decision authority subscales were high (0.79 and 0.84, respectively). Four items assess job demands. Cronbach’s alpha for this scale is reported as 0.67 [75] and in the 2001 study was 0.69.

Responses are required on a four point scale from, 1 = “Often”, to 4 = “Never”, to such questions as “I have a good deal of say in decisions about work”, and “Do you have to work very fast?” Twelve of the fifteen items in the job control scale are worded such that high control gives the lowest score – these are recoded and summed with the other three items to give a total score, where high score indicates high job control. In the job demand scale, three items are worded such that high job demand gives the lowest score. These are recoded and summed to the remaining questions, such that a high score indicates a high level of job demand.

In addition, the PATH study (from which the pilot study derived data on job control and demands) and the 2001 study included two questions on job security, one question on perceived fairness of pay, conditions and benefits, one question on number of hours worked in a routine week and one question asking how many days off work the respondent had had in the past month. These were used individually in the analysis.
All responses are self-reported and no attempt was made to verify the respondents’ scores by independent assessment of the job control or demands. Bosma et al [75] found only a modest correlation between self-reported and independently assessed job control (Pearson correlation coefficient 0.41), but both showed a strong relationship to new reports of coronary disease.

4.5.13 Hostility

Trait hostility is assessed by four items in the PANAS-X scale, as outlined above.

4.5.14 Self-esteem

One of the most widely used measures of self-esteem for many different areas of research is Rosenberg’s Self Esteem scale, published in 1965 [468]. This is a ten-item scale in which participants are asked to agree on a scale of 1 to 4 (Strongly agree to Strongly Disagree) with a number of statements, such as “I certainly feel useless at times”. Positively and negatively worded questions are included. The scale can be recorded as a total score (after reverse coding negatively worded items) out of 40 or it can be categorised into positive and negative self-esteem. The scale has excellent test-retest reliability and internal consistency [469]; in the 2001 study, the alpha coefficient was 0.85.

4.5.15 Mastery

Mastery, or a sense of personal control is measured in the PATH study and subsequently in the 2001 study using the Pearlin Personal Mastery Scale [470]. This is a 7-item scale requiring a response to a statement such as, “Sometimes I feel that
I’m being pushed around in life”, on a four point scale from 1 = “Strongly Agree” to 4 = “Strongly Disagree”. Positively and negatively worded statements are included.

After reverse coding of negatively worded statements and summing of the scores, a low score suggests a high level of control, while a high score suggests a low level of control. The scale has been widely used in health research [471] and has been shown to exhibit good construct validity and internal reliability [465]; in the 2001 study the alpha coefficient was 0.85.

4.5.16 Ruminative Style

Ruminative style is assessed by a 10-item scale, originally developed by Nolen-Hoeksema et al [472] to study the effect of this style of thinking on depression and stress after the Loma Pieta earthquake.

Participants respond to statements regarding their thoughts about themselves on a four-point scale, 1 = “Never”, to 4 = “Always”. Statements are of the form “I think about how alone I feel”, “I think about all my shortcomings, failings, faults and mistakes.” All items are worded such that a high score indicates a high degree of ruminative thinking. Scores are summed over all items. In the 2001 study, this scale had excellent internal consistency with a Cronbach’s alpha of 0.85.

4.5.17 Household Responsibility

Four questions were used in the PATH study and in the 2001 study to assess the level to which the participant assumed responsibility for different household tasks, such as childcare, financial management, housework and providing money for the household.
Participants answered questions such as, “To what extent are you responsible for childcare in your household?”, on a five point scale from “Fully responsible” to “Not at all responsible”. The responses were summed to give a total household responsibility score.

4.6 **Socioeconomic variables**

Socioeconomic status was assessed using a variety of measures, in an effort to see if these alternative indicators of SES performed differently in relation to the biological markers.

4.6.1 **Occupation**

Each participant’s occupation was assessed in the PATH study, with questions designed to allow coding to the Australian Standard Classification of Occupations, 2nd edition (ASCO, 2nd ed) [473]. Questions included, ‘job title’ and a description of the main duties and activities of that job. Basic information was sought on the nature of the employer (public service, own business, profit-making business, family business), the level of management responsibilities involved in the job and the size of the employing organisation. For those who were currently unemployed, the same information was sought on the last employment. Parents at home with children are assigned an ASCO code of 0999, i.e. they do not fit into the classification system, so that last employment was coded.

In the 2001 study, similar information was sought on the participant’s partner to enable exploration of a household SES by occupation as well as personal SES by occupation.
All occupations were coded to the ASCO, 2nd ed. using the ASCO II coding CD-ROM [474]. Any problems with coding were discussed with the ABS, for resolution. At the completion of coding, occupational data on a random sample of fifty participants was sent to the ABS for coding and checked against the coding for the 2001 study. There was agreement on codes for 80% of the occupations, with the remaining differences in coding making only minor alterations to occupational level and occupational status.

4.6.2 Occupational status

Once occupations had been coded to the four-digit ASCO code, a corresponding occupational status score was assigned to each participant using the ANU3_2 occupational status scale [475]. This converts each occupation (as coded to the ASCO II) to a scale from 1 to 100, which expresses the status of particular occupations as perceived by population samples. Examples of the extremes of the scale are medical specialists (occupational status 99.7), and domestic cleaners (occupational status 3.7). Incompletely described occupations, or non-paid occupations such as voluntary work cannot be assigned an ASCO code with the first figure between 1 and 9, or an occupational status.

Two occupational status codes were assigned to each participant. The first represented their personal occupational status based on their own occupational level. The second represented the highest occupational status in the household (respondent’s
or partner’s occupation) and is subsequently termed “household occupational status” or “household status”.

4.6.3 Education

Education was assessed in the PATH study with questions designed to allow coding to the Australian Bureau of Statistics Classification of Qualifications (ABSCQ), which was developed by the ABS for the 1991 census. The Australian Standard Classification of Education (ASCED) has recently superseded this scale.

The ABSCQ assesses the highest level of schooling completed and then the level of post-secondary or tertiary education completed, differentiating this into trade certificate, undergraduate diploma, bachelor’s degree, and higher degree. The ASCED codes levels of primary and secondary education more clearly than the ABSCQ, but the delineation of different certificates and diplomas as presented in the PATH questionnaire codes more clearly to the ABSCQ. Thus initial coding was to the ABSCQ, with subsequent conversion to the ASCED using published conversion sheets [476].

There are nine categories of educational attainment ranging from ‘1’ – higher degree, to ‘9’ – pre-primary education.

4.6.4 Income

Both personal and household income were measured in the Biomarkers Study. Categories for personal income are those used by the ABS in the 1996 Australian Census, except that a new highest income category was added, to spread those at the
top in relatively affluent Canberra. Similarly, the household income scale has additional high-income categories, to a maximum of $3,500 or more per week ($182,000 per year). All income is gross income from all sources, i.e. tax is not deducted, and interest, rents received etc are included. Household income includes the income of all persons who are part of the household – the participant, their partner, children and others who share food and essentials for living in the dwelling.

4.6.5 Number of cars

The number of registered motor vehicles is used as a measure of wealth. There are clearly some difficulties in the interpretation of this as a measure of SES. The number of cars in a family is affected not just by SES, but also by the family composition. Thus a high SES family may have a car for each member of the family who can drive, which may be two for a family of four that consists of parents and two toddlers, or four if the family consists of parents and two teenage or adult offspring.

In addition, while lack of any car may be an indicator of deprivation and hardship, it may also be an indicator of environmental awareness and a conscious decision not to be a vehicle-owner.

4.6.6 Accommodation

Type of accommodation provides another measure of wealth. In the 2001 study and the pilot study, participants were asked to indicate their current accommodation type, from a given list ranging from home ownership to rental housing to government housing to caravans etc.
4.6.7 Perceived place in the community and in Australia

In the 2001 study, a measure of where people see themselves in relation to others was included. This consisted of a picture of a ladder. Participants were asked to place themselves on the rung that depicted where they thought they stood at this time in their lives. In two separate questions, participants ranked themselves compared to their own community and to the Australian community as a whole. This format has been used to examine subjective social status [477], but specifies “standing” in the community, rather than SES. It is assumed, for the purposes of this thesis, that this is largely a self-rated measure of SES, although factors such as the importance of money, individual self-esteem etc may play a part in where people rank themselves on the ladders.

4.6.8 Childhood socioeconomic status

Childhood socioeconomic status was measured in both parts of the Biomarkers study by questions about parents’ occupation and education when the participant was aged 12.

**Education.** Parents’ education was assessed separately on a simple scale asking about the highest educational achievement, ranging from “no schooling” through “some primary schooling” to “completed high school”, “trade certificate” and “university degree”.

**Occupation.** Parents’ occupations were assessed by asking the job title and the job description for each parent, when the participant was aged 12. This occupational data was then coded to the ASCO 2nd edition, using the ASCO coder (as above), and an occupational status assigned in the same way as for “Occupational status” above.
Participants experienced some difficulty with the question regarding parent’s occupation. A few people read it as “the occupation of the parents when they themselves were 12” – usually answered as “they didn’t work when they were 12”; while others answered that their fathers were computer technicians or consultants – hardly likely thirty odd years ago!

4.7 Health variables

4.7.1 Physical

Self-rated health was assessed in the PATH study and in the 2001 study using the SF-12, which gives two global summary measures – physical health and mental health [478]. There is some disagreement as to whether the SF-12 adequately measures general physical and mental health in Australian populations, however it has recently been validated for use in Australia [479-481]. Taking any medications was recorded as Yes/No, and details of exact medication were listed.

4.7.2 Psychological

The SF-12 provides a general measure of mental health, including questions such as, “How much of the time during the past 4 weeks have you felt down?”

In addition, Goldberg’s scale provides a global measure of anxiety and depression [482]. The scale was adapted from a standardised psychiatric research interview and
designed to be used by non-psychiatrically trained personnel to detect psychiatric illness. It is an 18-item questionnaire requiring “Yes/No” responses to questions asking how the participant has been feeling in the past month, e.g. “Have you felt keyed up or on edge?”

4.8 Behavioural variables

4.8.1 Physical activity

Both frequency of physical activity (from “Never” to “3 times a week or more”) and total number of hours per week were measured in the PATH study and the 2001 study. Each of these required separate responses for mildly energetic, moderately energetic and vigorous activity.

From these responses, number of hours of exercise per week was converted to a weighted summary measure of physical activity using the working metabolic rate for each level of activity, to create a common metric [483]. Number of hours of mild activity is multiplied by 4, moderate activity by 6 and vigorous activity by 10 (representing metabolic equivalents, (METs)).

Mild activities, with an energy expenditure of 3-5 METs include brisk walking, golfing; moderate activities, with an energy expenditure of 5-7 METs include cross-country skiing, swimming and dancing with sufficient intensity to cause sweating and breathlessness; heavy exercise, with an energy expenditure of 7 or more METs includes jogging at a sufficient intensity to cause profound sweating and/or breathlessness.
The frequency of mild, moderate and vigorous activity were retained as separate variables.

4.8.2 Smoking

Smoking status was assessed with two questions, “Do you currently smoke?” and “How many cigarettes do you usually smoke in one week?”. Of those who did not currently smoke (87% of the sample), 39% had smoked in the past, but no further detail was elicited.

4.8.3 Alcohol intake

A single question from the PATH study was used to assess frequency of alcohol intake, from “not in the last year” to “four or more times per week”.

4.9 Biological variables

Blood was collected by Capital Pathology in the pilot study and by ACT Pathology in the 2001 study. All samples were taken fasting, and the majority were thus early morning samples.

4.9.1 Fibrinogen

Blood was collected in a citrated tube, then centrifuged to separate the plasma. Specimens were stored at 4°C and tested within 2 hours of collection. Estimation of the fibrinogen level uses the thrombin method, which relies on the principle that the thrombin clotting time of dilute plasma is inversely proportional to the fibrinogen concentration of the plasma. Thrombin clotting time is the rate of the reaction in
which soluble plasma fibrinogen is converted by the enzyme, thrombin, into insoluble fibrin. At high thrombin concentrations and low fibrinogen concentrations (as it is diluted in the test system), the reaction rate becomes a function of the fibrinogen concentration.

4.9.2 Serum lipids

The 2001 study and the pilot study measured total serum cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and triglycerides. Fibrinogen is associated with low levels of HDL and high levels of triglycerides, and these in turn may vary by SES and different health states. HDL and triglycerides are thus possible confounders of the relationship between fibrinogen and SES and fibrinogen and health.

In addition, total cholesterol and LDL are measured as a part of this battery of tests and provide useful information to feed back to participants.

Cholesterol measurement is performed using the Ortho Clinical Diagnostics dry slide technology Cholesterol slide.

4.9.3 Glycated haemoglobin

Glycated haemoglobin is measured by high performance liquid chromatography, from whole blood collected into an EDTA tube. The sample can be kept at 4º C for up to 7 days. Fasting serum glucose was measured to check for hyperglycaemia and to provide information on this possible confounder.
4.9.4 Serum neopterin

ACT Pathology performed the serum neopterin assay, using a commercial ELISA kit supplied by Brahms Diagnostica.

Serum for neopterin assay was collected by venipuncture in the standard way, into a light-protected tube (wrapped in aluminium foil), and refrigerated. The serum was separated and frozen at −20°C until assay, within six months. Due to a communication failure, the first specimens in the pilot study were not light protected, and other odd samples during the pilot study were not light protected for a variety of reasons. Lack of light protection was noted on the result form.

4.9.5 Salivary IgA

Saliva was collected by gentle suction using a suction pump (supplied by the Canberra Centre for Mucosal Immunology) and a disposable plastic catheter with a mucous trap. This ensured that no saliva contaminated the pump. (Details of saliva collection and analysis are contained in Appendix C)

Saliva was collected in the mucous trap, sealed and placed on ice for transportation. It was then frozen at −20°C within one hour and subsequently transferred to −80°C. Each saliva tube was labelled with the ID number, date and time of collection with an indelible marker. All samples were collected approximately two hours post-prandially, and participants had been asked not to eat or drink between the meal and the saliva collection. No stimulation was used to increase saliva flow.
Salivary IgA is detected by use of an antibody-sandwich ELISA technique. In essence, the wells of the ELISA plate are coated with unconjugated anti-IgA and allowed to incubate. This allows the anti-IgA to attach to the base and sides of the well. Excess anti-IgA is washed off and saliva (or standard) is then added to the well. The anti-IgA binds to the IgA in the saliva while it incubates. Excess saliva is washed off. Then a conjugated anti-IgA is added to the wells, which will in turn bind to the IgA that has already been bound to the unconjugated anti-IgA and therefore the well itself. The conjugated anti-IgA has an enzyme attached to it, which causes a colour change when certain chemicals are added to the mixture. After the conjugated anti-IgA has been allowed to incubate, excess is washed off and the appropriate chemical added. A colour change occurs that is proportional in optical density to the amount of enzyme (and thus conjugated anti-IgA bound to IgA) in the solution. This is compared to standards that have known levels of IgA and have been tested on the same plate, to give the IgA level.

A qualified technician at the laboratories of the Canberra Centre for Mucosal Immunology (CCMI), University of Canberra carried out all salivary IgA analyses.

### 4.9.6 Waist-hip ratio

**Waist measurement.** Participants were measured in light street clothing, without jumpers, coats or belts. They were requested to stand upright, feet together, stomach relaxed and hands at their sides. The waist measurement was then made at the end of normal expiration, at the narrowest point between the ribs and the hips as viewed from the front. The tape measure was adjusted several times to ensure a consistent
measure of the narrowest point was made. This is in keeping with the method of Boyle et al [301], chosen as a simple method that takes account of differing body shapes.

**Hip measurement.** As above, measurements were made over light street clothing with participants standing upright, feet together and hands at their sides. The hip measurement was measured at the point of maximal extension of the buttocks, when viewed from the side. Again several measurements were taken to ensure this was the maximum hip circumference and that the measurement was accurate. All measurements were recorded to the nearest half centimetre.

All measurements were taken along a horizontal plane with a plastic tape measure. The flexibility of the plastic tape measure was preferred over a metal tape measure, but the accuracy of the plastic measure was checked following every ten participants.

**4.9.7 Body mass index**

**Height.** Height was measured with the participant without shoes, standing with heels against the wall and using a stadiometer to ensure the correct angle of measurement at the top of the head. A metal tape measure was then used to measure from the floor to the bar of the stadiometer. There was modest variation in the surface that participants stood on, so that height was measured only to the nearest centimetre.

**Weight.** Participants were weighed on electronic scales in light street clothing without shoes, with weights recorded to the nearest 0.5 kg.
All height, weight, waist and hip measurements were taken by the same person to ensure consistency in measurement technique.

4.9.8 Blood pressure

Blood pressure (BP) was measured in the PATH study using an automatic blood pressure device. Because the participants in the pilot study had only recently participated in the PATH study, blood pressure was not re-checked and the reading from the PATH study was used in the analysis.

In the 2001 study, blood pressure was measured using a Speidel and Keller Iso-Stabil 5 anaeroid sphygmomanometer, with a standard and large cuff, and a Hewlett Packard stethoscope. Blood pressure was measured in the left upper arm, with the participant seated. A standard BP cuff was used unless this covered less than two-thirds of the distance from the elbow to the shoulder. A small cuff can lead to a spuriously high blood pressure measurement [484]. The systolic pressure was taken at the first phase of the Korotkoff’s sounds. The diastolic pressure was recorded as phase 5 of the Korotkoff’s sounds (when the sound disappears) except for the occasional person where the sounds continued down to zero. In this instance, the muffling of the sounds (phase 4) was taken as the recorded diastolic pressure. The sphygmomanometer gauge had 2mm Hg gradations, so that BP is recorded to the nearest 2mm Hg.

In the event that the blood pressure was elevated, (systolic greater than 150mmHg, diastolic greater than 95mm Hg) the participant rested (and usually did the saliva
sample) and a repeat blood pressure was taken 5-10 minutes later. The lower of these two blood pressure measurements was taken as the recorded blood pressure.

4.10 Data management

Separate Access databases for the pilot study and the 2001 study were maintained to record contact information, interview details, address changes etc as well as to record date of blood collection and date of saliva collection, date of final correspondence, and date of data entry. Data on all potential study participants were retained to aid in analysis of representativeness of the sample. All databases were password protected.

Data from the questionnaires, body and biological measurements were entered into Access databases using forms to minimize data entry errors. Limits were placed on acceptable values and acceptable data types (numeric, string, date etc). On completion of data entry, all data were transferred to Stata 7 files using Stat Transfer 6. Double data entry was performed for the pilot study to check on accuracy of data entry.

4.11 Data checking

Using Stata 7, data were checked initially by simple tabulation looking for unlikely values. These had been largely screened out by the use of limits on entered values in the Access database. Missing values were checked back against the original data. Further checks were carried out using cross-tabulation searching for impossible values, e.g. participants recorded as being non-smokers, who were recorded as smoking a certain number of cigarettes per day.
Categorical variables with categories containing only a few observations were collapsed. All missing values had been entered in Access as “99” and these were recoded to “.”, the missing variable code in Stata 7.

All variable scores were calculated in accordance with the original formulation of the scales. For those psychosocial scales in which the score was a sum of the individual items (after reverse coding when necessary), a single missing value was imputed as an average of the other items in the scale, i.e. a total for the scale was calculated, using the sum of the remaining items in the variable, divided by the number of remaining items and then multiplied by the total number of items in the scale to achieve a summary score.

For the assessment of the temporal stability of the markers, only the data from the pilot study were analysed, as this included, for each participant, measures of each marker taken on two occasions, three months apart. However, for the analysis of the performance of the markers and their relationship to psychosocial factors, the pilot data were aggregated with the data from the 2001 study to increase the sample size. The variables measured in the pilot study and in the 2001 study were similar, although some questions in the pilot study were omitted from the 2001 study and thus were not included in the subsequent statistical analysis.

In aggregating the data, PATH study data on the pilot subjects were used. Participants in the pilot study had been interviewed for the PATH study within the previous six months (in 2000). Comparison of scores on identical variables measured in the PATH
study and again in the 2001 study indicated that most variables had high test-retest reliability (intraclass correlations generally greater than 0.45) over twelve to eighteen months, suggesting that the use of PATH study data for the pilot participants was valid. (Details of this analysis are reported in Appendix E).

4.12 Data manipulation

For the purpose of this thesis, and the examination of the relationships between a number of psychosocial factors and several biological variables, the data were reduced using principal components analysis. In this way, factors representing groups of variables could be included in the analysis. The single factor with the largest eigenvalue was retained (unrotated), unless a second eigenvalue was of a similar magnitude. When more than one factor was retained, a varimax rotation was applied. Following principal components analysis, the weighting of each element of the retained factor(s) was examined to ensure that these retained some meaning, e.g., two coping factors were retained – one with the highest loadings from positive, problem-focused coping, the other with the highest loadings from negative coping strategies. This is in line with the coping literature. Specific details of factors retained are outlined in the following sections.

4.12.1 Social support

The original social support scale separates positive and negative interactions with family, partner and friends. This was collapsed to create two new variables encompassing total positive support and total negative interaction [485]. Principal components analysis, retaining separate single factors for positive and negative interaction confirmed that support from family, friends and partner loaded
approximately equally onto the single factors. Number of friends was retained as a separate variable.

4.12.2 Childhood adversity and parenting

Two summary measures of childhood adversity were derived. The first summed the number of defined adverse childhood events (verbal abuse, sexual abuse etc). Notably, only 5 participants out of 339 had a score greater than zero, so this variable was dropped from further analysis. The second summary score was created by summing responses relating to a more general assessment of the emotional milieu of participants’ childhoods, e.g. the presence of parental affection, conflict and drug use in the household.

4.12.3 Social capital

Social capital was measured with two questions in both studies, but sense of trust and belonging were measured in more detail only in the main study. For the purposes of the current analysis, the original two questions were retained as separate variables. A summary measure of trust and belonging was derived from principal component analysis of the trust and belonging items, with one factor retained.

4.12.4 Personality variables

Principal components analysis of the personality variables (extroversion, psychoticism, neuroticism, personal mastery, self-esteem, optimism, hostility, affect, ruminative style, behavioural inhibition and activation) resulted in the retention of two personality factors. In the first factor there is strong positive loading from self-esteem, sense of optimism, positive affect and locus of control with moderate positive
loading from extroversion. In addition there is strong negative loading from negative affect, hostility, neuroticism, ruminative style and behavioural inhibition. The second personality factor has a strong positive loading from responsiveness to rewards, fun-seeking, drive and extroversion with a moderate positive loading from hostility, negative affect and ruminative style. The first factor thus has high loadings from “positive” personality variables while the second contains more elements of typically “negative” personality, including some aspects of Type A personality type.

4.12.5 Perceived stress scale

This scale was included only in the main study and analysis is thus restricted to these participants.

4.12.6 Coping

The original Brief Cope Scale results were reduced to two factors using principal components analysis with varimax rotation. In the first of these the main loadings were from positive reframing, planning, active coping and use of emotional and instrumental support. This factor thus represents active, positive coping skills. The second factor was composed largely of disengagement, venting, denial and self-blaming, i.e. largely negative coping skills. These two factors were used in the analysis.

4.12.7 Work strain

Work strain variables include those assessing decision authority and skill discretion, which together constitute ‘job control’, and those assessing job demands. Each of job control and demand were split at the median to create four categories of job strain
(according to the Karasek model): high control with low demands, low control with high demands, low control and low demands, and high control with high demands. These were entered into the analysis as three separate dummy variables.

### 4.12.8 Household responsibility

Four questions assess the level of household responsibility assumed by the participant. One of these measures responsibility for childcare and thus is limited to those with children. An “average household responsibility” variable was obtained by summing scores across all variables and dividing by the number of variables included, to create comparable scores for those with and without children. A second variable was created to represent a total of the four household responsibility variables, assigning a value of 5 (0% responsible) to those with no children. This was then recoded so that a high score reflected a high level of responsibility in several different spheres of life, i.e. fully responsible for household tasks, childcare, financial management and providing money for the household.

### 4.12.9 Economic strain

The data from the pilot study were slightly different from those collected for the 2001 study as noted above. To maximize the use of the data and avoid large numbers of missing values, the extra questions from the 2001 study were summed and averaged as a separate variable, with a range of 1-4, where a high value denotes a high level of economic strain. Principal components analysis was used to create a single factor from the nine items common to both studies, with approximately equal loading from each item.
4.12.10 Occupation

All occupational variables were classified to the Australian Standard Classification of Occupation 2nd ed. as already outlined. Occupational variables included: subject’s occupation; partner’s occupation; mother’s occupation; father’s occupation. While occupations were coded to the four-figure or six-figure code (when there was sufficient information) this was used only to assign an occupational status value. In the statistical analysis only the first level occupational code was used, corresponding to the nine basic occupational groups.

Occupational status was assigned according to the ANU3_2 Occupational Status Scale, based on the four-figure ASCO code and retained as a continuous variable.

4.12.11 Education

Australian Standard Classification of Education coded educational codes were collapsed to single figure codes for use in the analysis. Parent’s educational achievement was measured on an ordinal scale from “no schooling” to “university degree” in seven categories.

4.12.12 Accommodation

Accommodation was measured as a categorical variable in the original data on an 8-point scale. In the analysis, the ordering of the variable was altered to make it ordinal, based on the expected association with socioeconomic status, i.e. own house/flat, rented house/flat, ACT Housing Trust/flat, living with relatives + other. Of only six participants in the last category, four were living with relatives, one was house-sitting and one was living in a workshop owned by the participant.
4.12.13 Equivalent income

Adjusted household income (equivalent income) was calculated based on gross weekly personal and household income, spouse’s occupation, number of children and their ages, and whether children lived at home part-time, full-time or not at all. Full details of the method are outlined in Appendix F.

4.12.14 Composite SES

A composite SES factor was derived from principal components analysis of measures of participants’ current SES. There was a strong loading on this factor from ‘personal income’ (0.32), ‘household income’ (0.35), ‘occupational status’ (0.41), ‘highest occupational status in the household’ (0.39), ‘educational level’ (0.31), ‘occupation’ (0.39) and ‘self-rated position in Australia’ (0.34). ‘Type of accommodation’ and ‘self-rated position in the community’ had weaker loadings (0.21 and 0.18 respectively), while the loading for ‘number of cars in the household’ was very low (0.02).

4.13 Missing values

There were few missing values, with good completion of the questionnaires and a good response rate to blood sampling. Comparison of those who completed the questionnaires with those who did not, revealed no statistically significant differences in biological measures or available SES measures. Comparison of those who had blood tests with those who did not revealed no significant differences between the groups (including on SES measures), except that those who did not complete the blood test had a significantly higher waist measurement and waist-hip ratio, and significantly lower total physical activity score, i.e. tended to be less healthy. These
people may have contributed significantly to abnormalities in biological measures. The restriction of the sample with complete data to a more healthy subset of the study sample may decrease the variance in the biological measurements and thus decrease the likelihood of finding significant results. These are just the people that we may be most likely to see relationships in, so that their loss is unfortunate, with a likely bias towards the null. Analyses were performed only on those with complete data for each part of the analysis.

4.14 Statistical analysis

In general, bivariate correlation tests were conducted to analyse the relationship between the marker and different measures of socioeconomic status, as well as between each psychosocial, behavioural, biological and health variable and SES. Mediation analysis was then used to examine the mediating effect of the behavioural, psychosocial, health and biological variables on the association between each marker and each measure of SES. This was followed by the construction of multiple linear regression models for the marker and SES, psychosocial, behavioural, health and biological factors. Month of blood or saliva collection was controlled for in all multivariate analyses. Age was not included in the models as the study sample was restricted to a narrow age range (40-44 years).

In some situations, summary variables, eg job strain, were included in the analysis in addition to the individual variables from which they were derived, eg job control, job demands. In this situation multiple collinearity is a problem, however both types of variables were initially included in the models and then repeated models examined
with and without each individual or summary variable to find the best model. In some situations, a model was obtained that included both individual and summary variables, with opposite signs for the coefficient. In such situations, the variables essentially cancel each other out and the model fit was not significantly different when both sets of variables were dropped.

4.14.1 Mediation analysis

My first two hypotheses are that the biomarkers vary with some measure of socioeconomic status and there is also variation in the psychosocial factors, with socioeconomic status. Having established this, the third hypothesis states that at least some of this co-variation can be ‘explained’ by the measured psychosocial factors. A number of statistical methods have been used to test the importance of variables that have associations to both the independent and the dependent variable and ‘mediate’ the relationship between them, i.e. $X \rightarrow I \rightarrow Y$, where $X$ is the independent variable, $I$ is the intervening or mediating variable and $Y$ is the outcome or dependent variable [486].

Mediation is distinct from effect modification in which the relationship between the dependent and the independent variables is different at different levels of a third variable. In mediation, an intervening variable is related to both the dependent and the independent variable and when this relationship is modeled, the direct relationship between the dependent and independent variables is weakened. The association between the $X$ and $Y$ variables may be completely or partially mediated by an intervening variable.
Note that mediation implies a causal hypothesis – an independent variable affects a mediator, which in turn influences a dependent variable. However, causality cannot be demonstrated from the analysis alone.

**Figure 4.1. Mediation model**

The indirect effect is given by $\tau$ and is the (unstandardised) coefficient of the regression equation containing only the dependent variable and the independent variable,

$$Y = \text{intercept}_1 + \tau X + \epsilon_1 \quad \text{(Equation 1)}$$

($\epsilon_1$ represents unexplained variability).

The direct effect is given by $\tau'$, which is the regression coefficient of the independent variable, in the full model containing the dependent variable, the independent variable and the mediator(s),

$$Y = \text{intercept}_2 + \tau' X + \beta M + \epsilon_2 \quad \text{(Equation 2)}$$
The mediated effect is then $\tau - \tau'$. This is mathematically identical to the product of the two regression parameters $\alpha$ and $\beta$, where $\alpha$ is the regression coefficient of the equation containing the mediating variable regressed on the independent variable,

$$M = \text{intercept3} + \alpha X + \varepsilon_3$$  \hspace{20pt} (Equation 3)

and $\beta$ is the regression coefficient of the mediating variable in the full model, (Equation 2). Thus the mediation depends on the extent to which the independent variable changes the mediator and the mediator changes the dependent variable.

In psychology, path analysis represents a type of mediation analysis, and mediation analysis was indeed derived from path analysis. Less formal/quantitative mediation analysis can be performed by examining the change in the regression coefficient before and after adjustment for a third variable in a regression model. Difficulties arise in the presence of time-varying covariates such as smoking, diet, exercise etc, where the time-dependent covariate may both affect and be affected by, the study exposure [130]. In the current study, the exposure of interest is socioeconomic status, the outcome a biological measure. It seems unlikely that smoking, diet, exercise etc would affect SES – rather any association of these covariates, with the study exposure and outcome would logically be as intermediate or confounding variables. McKinnon notes that mediation and confounding are identical statistically [487]. The distinction must be made on conceptual grounds.
Mediation analysis also allows the consideration of multiple intervening variables, and the contribution of each intervening variable to the total effect can be calculated as:

\[
\frac{\alpha_n \beta_n}{\tau}
\]

Figure 4.2 Multiple mediator model

However, two problems may arise using this method to calculate the proportion of the total effect mediated by any intervening variable:

1. If \( \tau \) is very small, the proportion of the total effect mediated can be greater than 1.
2. When the signs of $\alpha$, $\beta$, and $\tau$ are different, the proportion mediated may have a negative sign, which is difficult to interpret.

An alternative is to use significance testing to identify statistically significant mediating effects and this method was used in the Biomarkers Study.

A commonly used (but conservative) method to identify significant mediating effects is based on the testing of three hypotheses based on the regression equations. Firstly, there must be a significant direct effect of the independent variable on the dependent variable. Secondly, there must be a significant effect of the independent variable on the mediating variable and thirdly, the mediator must have a significant relationship with the dependent variable, when both the independent variable and the mediator are included in the regression equation (cited in [488]).

However, MacKinnon notes “it is possible for there to be mediation even when the program effect is insignificant. If some mediators reduce the problem behaviour (dependent variable) and others increase the problem behaviour (a suppressor effect), there may be a nonsignificant overall program effect when mediation actually exists” [489]. (Mediation analysis has been mainly used in the evaluation of preventive programs, e.g. for smoking cessation, to assess elements that mediate the program’s success or failure. Hence the term program refers to the independent or X variable).

Several authors have developed other methods to test the significance of the intervening variable effect, i.e. tests of the hypothesis that $\tau - \tau' = 0$ or $\alpha*\beta = 0$ using
a variety of assumptions [488]. In the subsequent analysis, I have used the method of Sobel [490] to produce a $z$ statistic, which is then compared to the standard normal distribution.

$$ z = \frac{\alpha \beta}{\sqrt{\sigma^2_{\beta^2} + \beta^2 \sigma^2_{\alpha}}} $$

### 4.15 Sources of error

#### 4.15.1 Confounding

Possible cofounders, including exercise levels, gender, serum lipids, fasting serum glucose and smoking status were measured and tested in the regression model.

#### 4.15.2 Selection bias

Participants were selected randomly within each SES group to minimise selection bias. The use of biological markers as outcome variables means that participants could not have been selectively recruited to achieve particular outcome variables. There were strict selection criteria to form the base SES groups, from which the study groups were then chosen randomly to provide samples representative of each occupational status grouping.

#### 4.15.3 Measurement bias

All blood and saliva measurements were collected at a distance from the analysing laboratories and were carried out using study ID numbers as identification, i.e. the laboratory had no knowledge of the study hypotheses or of the identity of the participants. All body measurements, including diastolic and systolic blood pressure were carried out by the same person in an effort to decrease measurement error.
Although there was access to the SES of the interviewed participants, once the study groups had been defined, no record was kept of the SES group status on the contact list and no attempt was made to ascertain the SES group status during the contact and interviewing phases of the study. However, many participants were visited in their homes and to some extent SES was obvious from the living circumstances of the participant. All measurements were objective, requiring no interpretation and following a strict methodological protocol, to minimise bias and error. The pilot study blood pressures were measured by a number of different observers in the process of interviewing for the PATH study, but comparison of the PATH blood pressures for main study participants with those taken as part of the 2001 study suggested that these were comparable, with a reasonable correlation (intraclass correlation (ICC) = 0.67 (95% CI 0.61-0.73) for systolic blood pressure; ICC = 0.58 (0.50-0.65) for diastolic blood pressure).

Interviewing was carried out on an area basis, starting in the outer north and moving to the centre of Canberra, then starting in the outer south and moving to the centre. This was done for convenience since a single interviewer was seeing all participants. To a certain extent there is a greater proportion of higher SES people in the inner suburbs and that may have meant that these people were seen in a different month than those in other suburbs, thus potentially biasing results for those markers that vary by season or month of collection. However, participants did not undertake the blood tests immediately they were seen and there was a large degree of crossover in the months in which people from the same suburb were seen and when the blood test was done. Measurement bias would have been present if certain SES groups were over
represented in any month of blood collection. However, analysis of SES for month of 

blood collection reveals that there is no statistically significant difference in SES 
group status (based on occupational status) or household income in participants 
having blood taken in different months.

4.15.4 Self-report bias

Self-report was used for all measures of the SES variables and the psychosocial 
variables. Self-report is always subject to bias and error.

Exposure: SES was determined by a number of different variables, which were highly 
correlated. Occupation was coded on the basis of detailed job characteristics, 
allowing little opportunity for non-deliberate error. Educational attainment is 
generally completed in early adulthood and there is little reason to expect differential 
recall across the study sample. Income could be subject to exaggeration or 
minimization, however the high correlation with the other SES measures suggests this 
has not occurred to any great extent (see Results).

Covariates: Self-report psychological scales were chosen that had proven temporal 
reliability. Smoking status is subject to inaccurate self-report, particularly in ex-
smokers [491] and may be a source of misclassification. Self-rated stress levels, 
number of friends, measures of social capital may be influenced by current or recent 
events and thus be unreliable indicators of the usual situation. There seems no reason 
to suspect that this misclassification would be differential.
4.16 **Sample size considerations**

The sample size of 60 per group (five groups) was chosen to deliver 90% power to detect a slope of 0.08 mmol/l fibrinogen per step of a 5-point socioeconomic scale, using a two-sided significance test at the 5% level. This value is derived from Markowe (1985) who reported an average difference of 0.34mmol/l between three groups based on job stress [78]. This gives a total sample size of 300. In the 2001 study, four groups of 75 persons were actually delineated, as occupational status separated more clearly into four, rather than five groups.

4.17 **Representativeness of the PATH sample**

Age-specific data from the 2001 Australian Census were used to compare the PATH and 2001 study populations to the general population of the Canberra/Queanbeyan region with respect to marital status, occupation, and employment status.

**Registered marital status:**

For the purposes of the comparison, those who stated in the PATH study that they were in a ‘de facto’ relationship were combined with the ‘never married’. The census data do not have a ‘de facto’ relationship category. For this age group it is likely that in the census those who describe a de facto relationship in the PATH study could describe themselves as “married”, “separated”, “divorced”, “widowed” or “never married”.

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Participants in the PATH study and the 2001 study are slightly more likely to be married than the general population of this region. There is a somewhat lower proportion of divorced people in both the PATH study and the 2001 study. If de facto relationships are included in the “never married” category, the proportions of never married are similar in the 2001 study, PATH study and Census data.

**Current occupation:**

The proportion of the population in each occupational group are very similar for the PATH study and the 2001 Census, but for males there is a much higher proportion of the population in the “Managers and Administrators” group in the PATH study, and slightly less in the “Intermediate Production and Transport workers” group.

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Census PATH-total</td>
<td>PATH-yes 2001 study</td>
</tr>
<tr>
<td>Married</td>
<td>67.6</td>
<td>74.2</td>
</tr>
<tr>
<td>Married + de facto</td>
<td>81.6</td>
<td>83.1</td>
</tr>
<tr>
<td>Separated</td>
<td>4.9</td>
<td>3.5</td>
</tr>
<tr>
<td>Divorced</td>
<td>9.6</td>
<td>5.5</td>
</tr>
<tr>
<td>Widowed</td>
<td>0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Never married</td>
<td>17.5</td>
<td>8.9</td>
</tr>
<tr>
<td>Never married + de facto</td>
<td>16.4</td>
<td>15.3</td>
</tr>
</tbody>
</table>
Table 4.2 Percentage in each occupational group

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Census - 2001</td>
<td>PATH total</td>
</tr>
<tr>
<td></td>
<td>PATH - yes</td>
<td>Census - 2001</td>
</tr>
<tr>
<td>Managers and Administrators</td>
<td>19.2</td>
<td>30.3</td>
</tr>
<tr>
<td>Professionals</td>
<td>29.0</td>
<td>27.3</td>
</tr>
<tr>
<td>Associate Professionals</td>
<td>16.1</td>
<td>18.3</td>
</tr>
<tr>
<td>Tradespersons and Related Workers</td>
<td>12.6</td>
<td>10.2</td>
</tr>
<tr>
<td>Advanced Clerical and Service Workers</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>Intermediate Clerical, Sales and Service Workers</td>
<td>9.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Intermediate Production and Transport Workers</td>
<td>6.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Elementary Clerical, Sales and Service Workers</td>
<td>3.5</td>
<td>2.6</td>
</tr>
<tr>
<td>Labourers and Related Workers</td>
<td>3.6</td>
<td>2.2</td>
</tr>
<tr>
<td>Inadequately described</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Not stated</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

PATH-total: refers to the total PATH 40-44 years sample
PATH-yes: refers to those in the PATH 40-44 years sample who expressed interest in participating in the Biomarkers Study

Current employment status:

The 2001 census age group is 35-44 years and is compared to the PATH study 40-44 years.

Table 4.3 Percentage of sample by employment status

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Census - 2001</td>
<td>PATH total</td>
</tr>
<tr>
<td></td>
<td>PATH - yes</td>
<td>Census - 2001</td>
</tr>
<tr>
<td>Employed full-time</td>
<td>73.7</td>
<td>89.0</td>
</tr>
<tr>
<td>Employed part-time</td>
<td>11.3</td>
<td>5.6</td>
</tr>
<tr>
<td>Unemployed</td>
<td>3.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Not in the labour force</td>
<td>6.9</td>
<td>2.3</td>
</tr>
</tbody>
</table>

PATH-total: refers to the total PATH 40-44 years sample
PATH-yes: refers to those in the PATH 40-44 years sample who expressed interest in participating in the Biomarkers Study
More of the PATH participants are engaged in full-time work than in the Census data, for both males and females. Fewer males are engaged in part-time work, are ‘unemployed’ or ‘not in the labour force’ in the PATH sample compared to the Census data. Fewer females are ‘not in the labour force’. Part-time workers and unemployed females are equally represented in the PATH sample and in the Census data.

The PATH sample is reasonably representative of the Canberra-Queanbeyan population, although participants are more likely to be in the labour force, employed full time, be married and be managers or administrators. Similarly, the PATH-yes group is similar to the total PATH study sample on these parameters. The 2001 study sample is not designed to be representative of the Canberra Queanbeyan population – rather it is deliberately engineered to create equal numbers of participants in groups defined by SES. However, since within the cutoffs of occupational status chosen to delineate the SES groups in the 2001 study, the sample was randomly chosen, the participants should be representative of their SES group, at least as defined by occupational status.

4.18 Response rates

In the PATH study, 64.6% of eligible people agreed to participate and were interviewed (n = 2530). Of these, 2042 expressed interest in participating in the Biomarkers study. However, 794 people were ineligible for participation in the Biomarkers study due to poor health or changed residence status (moved out of area). This left a total study population of 1248 people.
In the pilot study, of 64 people contacted, 60 people agreed to participate – a response rate of 94%. Of these, 100% returned the questionnaire and had a saliva sample taken on two occasions. 100% had the first blood test and 59 out of 60 had two blood tests, three months apart (98%). One participant was found to have diabetes on blood testing and was subsequently excluded from the analysis.

In the 2001 study a total of 349 possible participants were contacted but 24 of these were no longer eligible to participate, either because they had now moved out of area, or because they had now developed a major illness. Of the 325 who were eligible to participate, 302 were interviewed, a response rate of 93%.

Of the 23 people who were eligible to participate in the 2001 study but who subsequently declined participation, 35% were in the lowest SES group, 35% in the next, 26% in the next and 4.3% in the highest SES group. While there is clearly a higher rate of failure to participate in lower SES groups, the total number declining to participate was small. In addition, randomly selected individuals from the same SES group replaced those declining participation, so that each SES group was equally represented in the final study sample. It is possible that those declining to participate were systematically different from those participating, but the numbers are low and this is unlikely to bias the results.

Of those who consented to participate in the 2001 study, 96% completed and returned the first questionnaire, 94% completed and returned the second questionnaire, 95% of
participants completed the blood sampling and saliva samples were obtained from 100% of participants. However, two saliva samples were unable to be analyzed as the tubes broke during storage (99.3% of samples available for analysis).

The preceding sections have outlined in detail the recruitment process and the collection, management and manipulation of the data in preparation for specific analyses to test the study hypotheses. The following chapter outlines and presents the results of that analysis.
Chapter 5.

Results

5.1 Introduction

In this chapter, I firstly examine (in section 5.2) the various measures of socioeconomic status and the associations between them. The detail of the actual and derived measurements is outlined in Chapter 4. Previous studies have noted the high correlation between various measures of SES, but this is not absolute, and different measures may provide information on different aspects of the concept of SES. Thus, education is usually gained as a young person and may partly reflect childhood and early adulthood SES more than income and occupation which may be more purely a measure of current SES. Accommodation and cars are used as measures of wealth – cumulative, rather than current, SES, while subjective measures of SES are relatively new, based on the theory of the importance of relative, rather than absolute SES in the determination of health.

My first hypothesis states that psychosocial factors vary by socioeconomic status. The psychosocial hypothesis suggests that such variation is at least partially causative of the socioeconomic variation in ill-health. Yet few studies have specifically investigated whether such a co-variation occurs in the general population. A wide range of social and psychological factors was measured in this study, and their variation by SES is outlined. There is ample evidence that various behavioural practices differ by SES. For example, smoking and low levels of physical activity are
more common in lower SES groups. Such associations are also examined in this study population.

Kelly et al suggest that one necessary requirement of a biological marker of the association between SES and health is that it should be stable over time [154]. That is, a single measurement reflects the usual level of the marker; the level of the marker is not highly variable so that any single measurement is not subject to wide day-to-day fluctuations. In section 5.4 this is specifically examined using the results from the pilot study, where blood and saliva samples were collected on two occasions, three months apart.

Biological systems are interconnected and the psychosocial hypothesis as well as the wide range of health conditions that have an SES gradient, suggest that socioeconomic status may somehow cause a general susceptibility to ill-health. Previous research suggests that stressor exposure may have effects on the immune [461, 462], endocrine [158], and metabolic systems [265], so that parallel variations in markers of several different body systems might be expected. Bivariate correlations between the measured markers are explored in section 5.5.

In section 5.6, I examine each marker in turn, to look at the variation of the measured biological markers with the various measures of socioeconomic status (SES). The literature shows that mortality and morbidity vary by SES for most measures of both. This thesis investigates the period before morbidity might occur – the participants in this study were all healthy, with self-rated health “good”, “very good” or “excellent".
My second hypothesis is that there is a socioeconomic gradient of increasingly adverse biological profile (as indicated by these biological markers) in healthy adults. The bivariate associations between each marker and the measured behavioural and psychosocial variables are also explored to provide background for the subsequent mediation and multiple regression analysis.

My third hypothesis states that the socioeconomic variations in the biological variables are mediated by psychosocial factors. The mediating role of both behavioural and psychosocial variables on the association between each marker and each measure of SES is thus explored using mediation analysis. Finally, multiple regression models are constructed to examine the concurrent effects of socioeconomic, behavioural and psychosocial variables. Although this is a cross-sectional study and no causal inferences can be drawn, the models examine the relative (independent) contributions of various factors to the variation in each biomarker.

Table 5.1 presents a brief outline of some of the socioeconomic characteristics of the study participants (total of pilot and 2001 study).
Table 5.1. Socioeconomic characteristics of study participants.

<table>
<thead>
<tr>
<th></th>
<th>Male N (%)</th>
<th>Female N (%)</th>
<th>All N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Personal income ($ per week)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;500</td>
<td>17 (8.9)</td>
<td>56 (36.4)</td>
<td>73 (21.2)</td>
</tr>
<tr>
<td>500-999</td>
<td>55 (28.9)</td>
<td>42 (27.3)</td>
<td>97 (28.2)</td>
</tr>
<tr>
<td>1000-1499</td>
<td>66 (34.7)</td>
<td>36 (23.4)</td>
<td>102 (29.7)</td>
</tr>
<tr>
<td>1500-1999</td>
<td>29 (15.3)</td>
<td>14 (9.1)</td>
<td>43 (12.5)</td>
</tr>
<tr>
<td>≥2000</td>
<td>23 (12.1)</td>
<td>6 (3.9)</td>
<td>29 (8.4)</td>
</tr>
<tr>
<td>Total</td>
<td>190</td>
<td>154</td>
<td>344</td>
</tr>
<tr>
<td><strong>Household income ($ per week)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1000</td>
<td>35 (18.6)</td>
<td>27 (17.2)</td>
<td>62 (18.0)</td>
</tr>
<tr>
<td>1000-1999</td>
<td>76 (40.4)</td>
<td>71 (45.2)</td>
<td>147 (42.6)</td>
</tr>
<tr>
<td>2000-2999</td>
<td>56 (29.8)</td>
<td>39 (24.8)</td>
<td>95 (27.5)</td>
</tr>
<tr>
<td>≥3000</td>
<td>21 (11.2)</td>
<td>20 (12.7)</td>
<td>41 (11.9)</td>
</tr>
<tr>
<td>Total</td>
<td>188</td>
<td>157</td>
<td>345</td>
</tr>
<tr>
<td><strong>Occupational status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24</td>
<td>56 (28.0)</td>
<td>36 (22.4)</td>
<td>92 (25.5)</td>
</tr>
<tr>
<td>≥24 and &lt;50</td>
<td>42 (21.0)</td>
<td>48 (29.8)</td>
<td>90 (24.9)</td>
</tr>
<tr>
<td>≥51 and &lt;70</td>
<td>41 (20.5)</td>
<td>40 (24.8)</td>
<td>81 (22.4)</td>
</tr>
<tr>
<td>≥70</td>
<td>61 (30.5)</td>
<td>37 (23.0)</td>
<td>98 (27.2)</td>
</tr>
<tr>
<td>Total</td>
<td>200</td>
<td>161</td>
<td>361</td>
</tr>
</tbody>
</table>

5.2 Correlation of socioeconomic variables

Table 5.2 presents the Pearson and Spearman correlation coefficients for different measures of current and childhood socioeconomic status. Pearson correlation coefficients are given for continuous variables, while Spearman’s rho is calculated for ordinal categorical variables.

Most measures of SES in this study are highly inter-correlated. The correlation of equivalent income and household income, and of occupational status and occupation are clearly the result of one being derived from the other. There is, however, a strong correlation between occupational and income variables, between education and income variables and between occupation and education variables. Interestingly, the correlation between current income and occupational variables is generally higher.
than between either of these and education. Education, in turn, correlates more highly with elements of childhood SES (parental occupation and education) than do measures of current personal SES (occupation and income). This supports the notion that education carries an element of childhood and early adult SES since most education is obtained in these periods of life.

Accommodation type has a relatively low, but statistically significant correlation with income and occupational variables, and only a marginally statistically significant association with education. Notably 85.4% of participants owned their own accommodation (compared to 66.0% in the Census 2001 for this region) with 9.7% in rental accommodation (21.5% in the Census) and 3.1% in government housing (8.0% in Census 2001). The remaining 1.7% were in some other type of accommodation (living with relatives, house-sitting etc). Accommodation type thus provides little variation compared to other measures of SES.

The number of cars in the household is poorly correlated with most other measures of SES. As noted in Chapter 4, number of cars in the household may be more related to household composition than SES in this study sample. In fact, the number of cars may represent a reverse measure of SES, since those who married younger and had larger families would now (in their forties) have teenage families. Those who married later or delayed childbearing (more common in higher SES groups) may now have only young children and no more than two cars. In addition, not having a car may not be a measure of low SES and hardship – rather it may be a lifestyle decision, by educated
Table 5.2 Correlation between socioeconomic variables - Female below diagonal; male above diagonal

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Equivalent income</td>
<td>1.00</td>
<td>0.57*</td>
<td>0.65*</td>
<td>0.16</td>
<td>0.49*</td>
<td>0.38*</td>
<td>0.41*</td>
<td>0.47*</td>
<td>0.44*</td>
<td>-0.09</td>
<td>0.11</td>
<td>0.09</td>
<td>0.06</td>
<td>0.20</td>
<td>0.68*</td>
</tr>
<tr>
<td>2. Personal income</td>
<td>0.62*</td>
<td>1.00</td>
<td>0.78*</td>
<td>0.15</td>
<td>0.64*</td>
<td>0.52*</td>
<td>0.47*</td>
<td>0.55*</td>
<td>0.47*</td>
<td>0.05</td>
<td>0.22*</td>
<td>0.22*</td>
<td>0.20*</td>
<td>0.26</td>
<td>0.74*</td>
</tr>
<tr>
<td>3. Household income</td>
<td>0.68*</td>
<td>0.54*</td>
<td>1.00</td>
<td>0.27</td>
<td>0.68*</td>
<td>0.40*</td>
<td>0.48*</td>
<td>0.53*</td>
<td>0.56*</td>
<td>0.20*</td>
<td>0.29*</td>
<td>0.10</td>
<td>0.13</td>
<td>0.27</td>
<td>0.76*</td>
</tr>
<tr>
<td>4. Perceived position in community</td>
<td>0.17</td>
<td>0.03</td>
<td>0.17*</td>
<td>1.00</td>
<td>0.47*</td>
<td>0.12</td>
<td>0.23</td>
<td>0.24</td>
<td>0.27</td>
<td>0.03</td>
<td>0.06</td>
<td>0.11</td>
<td>0.13</td>
<td>0.14</td>
<td>0.26</td>
</tr>
<tr>
<td>5. Perceived position in Australia</td>
<td>0.45*</td>
<td>0.44*</td>
<td>0.49*</td>
<td>0.47*</td>
<td>1.00</td>
<td>0.41*</td>
<td>0.50*</td>
<td>0.54*</td>
<td>0.50*</td>
<td>0.08</td>
<td>0.26*</td>
<td>0.19</td>
<td>0.23*</td>
<td>0.22</td>
<td>0.67*</td>
</tr>
<tr>
<td>6. Education level</td>
<td>0.40*</td>
<td>0.49*</td>
<td>0.34*</td>
<td>0.32*</td>
<td>0.42*</td>
<td>1.00</td>
<td>0.58*</td>
<td>0.64*</td>
<td>0.62*</td>
<td>0.10</td>
<td>0.11</td>
<td>0.28*</td>
<td>0.34*</td>
<td>0.42*</td>
<td>0.73*</td>
</tr>
<tr>
<td>7. Occupational code</td>
<td>0.52*</td>
<td>0.54*</td>
<td>0.50*</td>
<td>0.21</td>
<td>0.35*</td>
<td>0.59*</td>
<td>1.00</td>
<td>0.85*</td>
<td>0.78*</td>
<td>0.16</td>
<td>0.25*</td>
<td>0.12</td>
<td>0.20*</td>
<td>0.30*</td>
<td>0.85*</td>
</tr>
<tr>
<td>8. Occupational status</td>
<td>0.50*</td>
<td>0.56*</td>
<td>0.50*</td>
<td>0.21</td>
<td>0.45*</td>
<td>0.64*</td>
<td>0.89*</td>
<td>1.00</td>
<td>0.91*</td>
<td>-0.11</td>
<td>0.20*</td>
<td>0.19*</td>
<td>0.28*</td>
<td>0.33*</td>
<td>0.91*</td>
</tr>
<tr>
<td>9. Household occupational status</td>
<td>0.46*</td>
<td>0.47*</td>
<td>0.62*</td>
<td>0.25</td>
<td>0.44*</td>
<td>0.56*</td>
<td>0.72*</td>
<td>0.81*</td>
<td>1.00</td>
<td>-0.07</td>
<td>0.21*</td>
<td>0.18</td>
<td>0.26*</td>
<td>0.32*</td>
<td>0.89*</td>
</tr>
<tr>
<td>10. Number of cars</td>
<td>0.03</td>
<td>0.05</td>
<td>0.27*</td>
<td>0.09</td>
<td>0.06</td>
<td>0.06</td>
<td>0.03</td>
<td>0.04</td>
<td>-0.02</td>
<td>1.00</td>
<td>0.03</td>
<td>-0.05</td>
<td>-0.07</td>
<td>-0.06</td>
<td>-0.07</td>
</tr>
<tr>
<td>11. Accommodation type</td>
<td>0.36*</td>
<td>0.25*</td>
<td>0.41*</td>
<td>0.11</td>
<td>0.30*</td>
<td>0.19</td>
<td>0.28*</td>
<td>0.30*</td>
<td>0.35*</td>
<td>0.18</td>
<td>1.00</td>
<td>-0.10</td>
<td>0.00</td>
<td>0.10</td>
<td>0.24*</td>
</tr>
<tr>
<td>12. Mother’s education</td>
<td>0.13</td>
<td>0.04</td>
<td>0.10</td>
<td>0.13</td>
<td>0.11</td>
<td>0.20</td>
<td>0.17</td>
<td>0.17</td>
<td>0.12</td>
<td>-0.15</td>
<td>-0.11</td>
<td>1.00</td>
<td>0.54*</td>
<td>0.31*</td>
<td>0.19*</td>
</tr>
<tr>
<td>13. Father’s education</td>
<td>0.18</td>
<td>0.08</td>
<td>0.20</td>
<td>0.08</td>
<td>0.14</td>
<td>0.15</td>
<td>0.16</td>
<td>0.18</td>
<td>0.15</td>
<td>-0.09</td>
<td>0.01</td>
<td>0.47*</td>
<td>1.00</td>
<td>0.47*</td>
<td>0.26*</td>
</tr>
<tr>
<td>14. Father’s occupational status</td>
<td>0.16</td>
<td>0.22</td>
<td>0.24</td>
<td>0.10</td>
<td>0.16</td>
<td>0.33*</td>
<td>0.32*</td>
<td>0.33*</td>
<td>0.35*</td>
<td>-0.06</td>
<td>0.11</td>
<td>0.35*</td>
<td>0.44*</td>
<td>1.00</td>
<td>0.37*</td>
</tr>
<tr>
<td>15. Composite SES</td>
<td>0.73*</td>
<td>0.74*</td>
<td>0.74*</td>
<td>0.25</td>
<td>0.56*</td>
<td>0.72*</td>
<td>0.87*</td>
<td>0.90*</td>
<td>0.85*</td>
<td>-0.01</td>
<td>0.39*</td>
<td>0.17</td>
<td>0.18</td>
<td>0.36*</td>
<td>1.00</td>
</tr>
</tbody>
</table>
inner city couples who have little need for a car (close proximity to work and shopping and no children) and are conscious of the environmental effects of cars (lack of physical exercise, air and noise pollution).

Such an interpretation is given added weight by inspection of the correlation matrix. Number of cars has a weak (non-significant) positive correlation with increasing SES for most measures. However there is a negative correlation with education, occupation and occupational status. In addition, although there is a weak positive correlation with personal income and particularly household income, when household income is adjusted for number of people in the household, the correlation becomes negative, i.e. those with higher equivalent income have fewer cars. The stronger correlation between household income and number of cars may reflect a larger household, rather than higher SES.

Perceived position in the community also correlates poorly with the more objective measures of SES (income, occupation, education). Possibly participants interpreted this “position” as something other than an SES position, e.g. general status, being well-thought of, or participating in the community.

By contrast, perceived position in Australia has a much higher correlation with education, occupational and income variables. This is perhaps a reflection of the perception that Canberrans are well-off compared to the rest of Australia - even those who do not
consider themselves particularly well-off within their own Canberra community, perceive that, compared to the rest of Australia, they are higher up the SES ladder.

Measures of parental socioeconomic status have a high intercorrelation, but a weaker correlation with measures of current SES. There is a modest correlation between father’s occupational status and the participant’s household income and occupational status. Parents’ educational achievement has a statistically significant correlation with participant’s education and current occupational level.

5.3 Socioeconomic variation of psychosocial, behavioural, biological and health factors

5.3.1 Psychosocial factors

In correlation analyses, the composite SES factor showed a strong negative correlation with a sense of economic strain \( r = -0.44, p<0.001 \), and a high positive correlation with job demands \( r = 0.45, p<0.001 \) and job control (skill discretion \( r = 0.50, p<0.001 \); decision authority \( r = 0.37, p<0.001 \)).

Weaker, but statistically significant (at \( p<0.05 \)), correlations existed with positive, active coping style \( r = 0.28 \), sense of optimism \( r = 0.24 \), social capital \( r = 0.26 \), job security \( r = 0.17 \), job marketability \( r = 0.16 \), number of hours worked per week \( r = 0.35 \), sense of belonging \( r = 0.22 \), total number of adverse life events \( r = -0.13 \), and positive interaction with friends and family \( r = 0.20 \).
Thus, with higher SES there is a greater sense of the ease with which participants could get a job at the same pay level and hours as their current job. Higher SES is also associated with greater number of hours worked per week as well as increased job demands, job control and job security. Lower SES persons in this sample are less optimistic, use less positive and active coping styles, have a weaker sense of belonging to their community and had more major life events.

Anxiety, depression and personality variables such as neuroticism, psychoticism, hostility, extroversion, locus of control and ruminative style did not have a statistically significant correlation with SES in this study sample. In addition there was no statistically significant correlation between SES (as measured by this composite factor) and negative interaction with family and friends, self-esteem, perceived stress or mental health as assessed by the SF-12.

Separate analyses by gender indicated that the magnitude and direction of effect was similar for men and women (see Table 5.3).

5.3.2 Biological and health variables

Using the composite SES factor, there was a weak but statistically significant correlation between lower SES and poorer self-rated health (Spearman’s rho = 0.13), and physical health as assessed by the SF-12 (r = 0.11). This pattern is stronger in males than females.
Table 5.3 Socioeconomic variation of psychosocial factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Economic strain</td>
<td>r = -0.50</td>
<td>r = -0.40</td>
<td>r = -0.44; p &lt;0.001</td>
</tr>
<tr>
<td>Job demands</td>
<td>r = 0.43</td>
<td>r = 0.45</td>
<td>r = 0.45; p &lt;0.001</td>
</tr>
<tr>
<td>Job control</td>
<td>r = 0.24</td>
<td>r = 0.28</td>
<td>r = 0.26; p &lt;0.001</td>
</tr>
<tr>
<td>Skill discretion</td>
<td>r = 0.39</td>
<td>r = 0.60</td>
<td>r = 0.50; p &lt;0.001</td>
</tr>
<tr>
<td>Decision Authority</td>
<td>r = 0.26</td>
<td>r = 0.46</td>
<td>r = 0.37; p &lt;0.001</td>
</tr>
<tr>
<td>Active coping</td>
<td>r = 0.35</td>
<td>r = 0.25</td>
<td>r = 0.28; p &lt;0.001</td>
</tr>
<tr>
<td>Passive coping</td>
<td>r = -0.21</td>
<td>r = 0.004</td>
<td>r = -0.13; p =0.02</td>
</tr>
<tr>
<td>Sense of optimism</td>
<td>r = 0.20</td>
<td>r = 0.28</td>
<td>r = 0.24; p &lt;0.001</td>
</tr>
<tr>
<td>Social capital*</td>
<td>r = 0.23</td>
<td>r = -0.29</td>
<td>r = 0.26; p &lt;0.001</td>
</tr>
<tr>
<td>Job security*</td>
<td>r = 0.15</td>
<td>r = 0.20</td>
<td>r = 0.17; p =0.002</td>
</tr>
<tr>
<td>Job marketability*</td>
<td>r = -0.20</td>
<td>r = -0.10</td>
<td>r = -0.16; p =0.005</td>
</tr>
<tr>
<td>Work hours/week</td>
<td>r = 0.23</td>
<td>r = 0.47</td>
<td>r = 0.35; p &lt;0.001</td>
</tr>
<tr>
<td>Sense of belonging</td>
<td>r = 0.19</td>
<td>r = 0.26</td>
<td>r = 0.22; p &lt;0.001</td>
</tr>
<tr>
<td>Life events</td>
<td>r = -0.21</td>
<td>r = -0.04</td>
<td>r = -0.13; p =0.01</td>
</tr>
<tr>
<td>Positive interaction</td>
<td>r = 0.16</td>
<td>r = 0.25</td>
<td>r = 0.20; p &lt;0.001</td>
</tr>
<tr>
<td>BAS reward</td>
<td>r = 0.07</td>
<td>r = 0.12</td>
<td>r = 0.08; p =0.19</td>
</tr>
<tr>
<td>BAS drive</td>
<td>r = 0.09</td>
<td>r = 0.16</td>
<td>r = 0.14; p =0.02</td>
</tr>
<tr>
<td>BAS fun</td>
<td>r = -0.03</td>
<td>r = 0.09</td>
<td>r = 0.04; p =0.47</td>
</tr>
<tr>
<td>Anxiety</td>
<td>r = 0.06</td>
<td>r = 0.01</td>
<td>r = 0.03; p =0.55</td>
</tr>
<tr>
<td>Depression</td>
<td>r = -0.07</td>
<td>r = -0.03</td>
<td>r = -0.06; p =0.25</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>r = 0.02</td>
<td>r = -0.09</td>
<td>r = -0.04; p =0.50</td>
</tr>
<tr>
<td>Psychoticism</td>
<td>r = -0.08</td>
<td>r = 0.06</td>
<td>r = 0.01; p =0.81</td>
</tr>
<tr>
<td>Hostility</td>
<td>r = -0.08</td>
<td>r = -0.18</td>
<td>r = -0.12; p =0.05</td>
</tr>
<tr>
<td>Extroversion</td>
<td>r = -0.004</td>
<td>r = 0.15</td>
<td>r = 0.06; p =0.33</td>
</tr>
<tr>
<td>Ruminative style</td>
<td>r = -0.09</td>
<td>r = 0.02</td>
<td>r = -0.05; p =0.38</td>
</tr>
<tr>
<td>Negative interaction</td>
<td>r = 0.03</td>
<td>r = 0.01</td>
<td>r = 0.02; p =0.69</td>
</tr>
<tr>
<td>Self esteem</td>
<td>r = 0.08</td>
<td>r = 0.04</td>
<td>r = 0.07; p =0.25</td>
</tr>
<tr>
<td>Perceived stress</td>
<td>r = -0.03</td>
<td>r = -0.03</td>
<td>r = -0.04; p =0.53</td>
</tr>
<tr>
<td>Mental health</td>
<td>r = -0.06</td>
<td>r = -0.002</td>
<td>r = -0.03; p =0.56</td>
</tr>
</tbody>
</table>

* Spearman’s rho

There was no correlation between weight and SES in men, but a weak correlation in women (r =-0.17). Of the biological measures, there was no statistically significant correlation between SES and diastolic blood pressure (r = -0.03) or systolic blood pressure (r = -0.03) in either sex. In men there was a weak SES correlation with serum
cholesterol (r = -0.16), but there was no significant SES correlation in either sex with serum triglycerides (r = -0.03), serum HDL cholesterol (r = -0.01), serum LDL cholesterol (r = -0.07). These findings are summarized in Table 5.4.

Table 5.4 Socioeconomic variation of biological and health variables

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical health</td>
<td>r = 0.20</td>
<td>r = 0.01</td>
<td>r = 0.11; p = 0.05</td>
</tr>
<tr>
<td>Self rated health*</td>
<td>r = 0.14</td>
<td>r = 0.14</td>
<td>r = 0.13; p = 0.02</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>r = -0.02</td>
<td>r = -0.12</td>
<td>r = -0.03; p = 0.60</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>r = -0.06</td>
<td>r = -0.09</td>
<td>r = -0.03; p = 0.52</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>r = -0.16</td>
<td>r = -0.05</td>
<td>r = -0.09; p = 0.11</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>r = -0.14</td>
<td>r = -0.03</td>
<td>r = -0.03; p = 0.65</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>r = -0.06</td>
<td>r = 0.14</td>
<td>r = -0.01; p = 0.80</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>r = -0.08</td>
<td>r = -0.14</td>
<td>r = -0.07; p = 0.20</td>
</tr>
<tr>
<td>BMI</td>
<td>r = 0.00</td>
<td>r = -0.24</td>
<td>r = -0.10; p = 0.07</td>
</tr>
<tr>
<td>Weight</td>
<td>r = 0.02</td>
<td>r = -0.17</td>
<td>r =0.009; p = 0.87</td>
</tr>
</tbody>
</table>

* Spearman’s rho

5.3.3 Behavioural measures

The association between SES and mild or moderate physical activity was not statistically significant, but there was a statistically significant positive correlation in men with the frequency of vigorous physical activity (r = 0.21, p = 0.004). Alcohol consumption was significantly correlated with SES in men and women (r = 0.30, p<0.001). Note that the item measuring frequency of consuming alcohol measured the range from “Not in the last year”, to “four or more times a week”; higher SES is associated with a level of alcohol intake that most people would consider healthy (less than a glass of wine with dinner each night) rather than excessive. In addition there was a statistically significant negative correlation with current smoking status in men (r = -0.34, p<0.001). These findings are summarized in Table 5.5.
Table 5.5 Socioeconomic variation of behavioural variables

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical activity</td>
<td>r = -0.21</td>
<td>r = -0.21</td>
<td>r = -0.19; p &lt; 0.001</td>
</tr>
<tr>
<td>Freq mild phys. activity*</td>
<td>r = 0.04</td>
<td>r = -0.01</td>
<td>r = 0.02; p = 0.68</td>
</tr>
<tr>
<td>Freq mod phys. activity *</td>
<td>r = 0.14</td>
<td>r = 0.05</td>
<td>r = -0.05; p = 0.33</td>
</tr>
<tr>
<td>Freq vig phys. activity *</td>
<td>r = 0.21</td>
<td>r = -0.09</td>
<td>r = -0.16; p = 0.33</td>
</tr>
<tr>
<td>Alcohol intake*</td>
<td>r = 0.25</td>
<td>r = 0.35</td>
<td>r = 0.30; p &lt; 0.001</td>
</tr>
<tr>
<td>Smoking status*</td>
<td>r = -0.34</td>
<td>r = -0.14</td>
<td>r = -0.25; p &lt; 0.001</td>
</tr>
</tbody>
</table>

(*Spearman’s rho)

5.4 Temporal stability of biomarkers (pilot study)

Intraclass correlation was performed for biological measures sampled on two occasions, three months apart (n = 59):

Table 5.6. Intraclass correlations for biological measures.

<table>
<thead>
<tr>
<th></th>
<th>Intraclass correlation</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>0.32</td>
<td>0.09-0.55</td>
</tr>
<tr>
<td>HbA1c</td>
<td>0.86</td>
<td>0.79-0.92</td>
</tr>
<tr>
<td>Salivary IgA</td>
<td>0.35</td>
<td>0.13-0.58</td>
</tr>
<tr>
<td>Neopterin</td>
<td>0.41</td>
<td>0.20-0.62</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.82</td>
<td>0.74-0.90</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.84</td>
<td>0.77-0.92</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>0.86</td>
<td>0.80-0.93</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.90</td>
<td>0.85-0.95</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>0.79</td>
<td>0.69-0.88</td>
</tr>
</tbody>
</table>

5.4.1 Fibrinogen

The intraclass correlation (ICC) for plasma fibrinogen measured at two time points three months apart was low at 0.32 (95% CI 0.09-0.55). Further investigation was undertaken to examine whether this low ICC was due to blood collections being taken at different times of the year, for different participants. Previous research has suggested that fibrinogen levels tend to be higher in blood collected in late winter/early spring [213].
The first blood samples were collected from participants in the pilot study from July to October (winter to spring), with second collections from November to January (summer). Figure 5.1a suggests lower fibrinogen levels at the second collection than the first, with the low ICC relating to the increase in fibrinogen from first collection to second collection for a few participants, while for most participants, fibrinogen decreased between the first and second collections.

**Figure 5.1a Fibrinogen level by collection time.**

In a regression model with “change in fibrinogen”, (fibrinogen (time1) – fibrinogen (time2), since plasma fibrinogen is generally higher at time1 than time2) as the dependent variable and the month of the first blood collection as the explanatory variable there was a significant difference in “change in fibrinogen” according to the month of first blood collection, with blood first collected in July and August showing a higher mean change than that first collected in September and October (July 0.48, August 0.52, September
0.11, October 0.07). Inclusion of month of second collection and number of months between collections did not improve the model. Thus, blood collected in July and August tends to have higher levels of plasma fibrinogen than that collected in other months and the difference in month of first blood collection may account for part of the poor temporal stability.

Standardization of fibrinogen levels to the mean (by subtracting the mean for each month from each sample taken in that month) for each collection month resulted in a small improvement of the ICC to 0.41 (95% CI 0.19-0.62). Figure 5.1b presents standardised plasma fibrinogen at each time point.

Figure 5.1b Standardised fibrinogen by collection time
When plasma fibrinogen levels for the 2001 study were analysed by month of blood collection, there was an apparent pattern over one year:

**Figure 5.2.** Mean and 95% confidence intervals of log plasma fibrinogen by month of blood collection (2001, using a cubic spline smoother).

In the 2001 study data, there appears to be a winter peak in values and perhaps a smaller mid-summer peak, with lowest levels in spring. Summary statistics for plasma fibrinogen by month are presented in Table 5.7.
Table 5.7. Plasma fibrinogen by month of blood collection (2001 study and pilot study)

<table>
<thead>
<tr>
<th></th>
<th>2001 study</th>
<th></th>
<th>Pilot study</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Median (range)</td>
<td>n</td>
<td>Median (range)</td>
</tr>
<tr>
<td>Jan</td>
<td>11</td>
<td>2.4 (2.0-2.8)</td>
<td>6</td>
<td>2.35 (1.5-3.5)</td>
</tr>
<tr>
<td>Feb</td>
<td>9</td>
<td>2.3 (1.6-2.6)</td>
<td>5</td>
<td>2.7 (2.3-3.0)</td>
</tr>
<tr>
<td>Mar</td>
<td>1</td>
<td>1.8</td>
<td>2</td>
<td>2.0 (1.8-2.2)</td>
</tr>
<tr>
<td>Apr</td>
<td>2</td>
<td>2.0 (1.7-2.3)</td>
<td>2</td>
<td>2.6 (2.3-2.9)</td>
</tr>
<tr>
<td>May</td>
<td>1</td>
<td>2.8</td>
<td>3</td>
<td>2.2 (2.1-2.3)</td>
</tr>
<tr>
<td>Jun</td>
<td>12</td>
<td>2.3 (1.9-3.9)</td>
<td>8</td>
<td>2.15 (1.9-2.6)</td>
</tr>
<tr>
<td>Jul</td>
<td>6</td>
<td>2.5 (2.0-2.9)</td>
<td>3</td>
<td>2.5 (2.1-2.8)</td>
</tr>
<tr>
<td>Aug</td>
<td>22</td>
<td>2.55 (1.7-4.7)</td>
<td>15</td>
<td>2.1 (1.1-4.1)</td>
</tr>
<tr>
<td>Sep</td>
<td>12</td>
<td>2.3 (1.8-2.6)</td>
<td>13</td>
<td>2.4 (1.7-3.9)</td>
</tr>
<tr>
<td>Oct</td>
<td>24</td>
<td>2.2 (1.3-3.3)</td>
<td>31</td>
<td>2.4 (1.2-4.2)</td>
</tr>
<tr>
<td>Nov</td>
<td>36</td>
<td>2.1 (1.7-4.0)</td>
<td>23</td>
<td>2.4 (1.6-3.1)</td>
</tr>
<tr>
<td>Dec</td>
<td>18</td>
<td>2.3 (1.9-3.2)</td>
<td>14</td>
<td>2.55 (1.2-3.9)</td>
</tr>
</tbody>
</table>

Plasma fibrinogen varied significantly according to the month in which blood was collected with no significant difference in this variation between males and females. Month of blood collection was retained in subsequent analyses to adjust for this effect.

In addition, plasma fibrinogen differed by which laboratory analysed the blood sample, with those in the pilot study having statistically significantly higher fibrinogen levels for
each month, in each gender. For this reason, the remainder of the analysis for plasma fibrinogen was carried out separately for the pilot study and the 2001 study.

5.4.2 Glycated Haemoglobin

The intraclass correlation for glycated haemoglobin values collected on two occasions three months apart was high at 0.86 (95% CI 0.79-0.92). Despite this, there was a significant variation in levels of glycated haemoglobin depending on the month of blood collection, with a peak in May/June and lowest levels in February/March. This has been previously noted by Schuck who observed a peak in the northern hemisphere in February and a nadir in August [244].

Figure 5.3. Variation in blood glycated haemoglobin by month of blood collection (mean ± SE mean, cubic spline smoothing curve)
In addition, there was a statistically significant difference between measured levels of glycated haemoglobin in the pilot study and in the 2001 study. The two sets of samples were collected and analysed by two different pathology providers and those in the pilot study were significantly higher than those in the 2001 study (pilot: median = 5.8; range = 5.05-6.8; 2001 study: median = 4.6; range = 3.6-5.9). Indeed the quoted normal range is wider from the initial pathology provider (4.3-6.4% compared to (2001 study) 4.5-6.0%). Variability between laboratories is a recognised problem with measurement of glycated haemoglobin [242]. The difference between the two samples could not be accounted for by month of blood collection, or any systematic difference in the participants in the two samples. The remainder of the analysis is undertaken only on the 2001 study sample in view of the small sample size of the pilot study.

**5.4.3 Waist-hip ratio**

WHR was considered to be a stable measure and was not measured on two occasions in the pilot study. A substantial change in WHR over a three-month period is unlikely.

**5.4.4 Neopterin**

The intraclass correlation for neopterin samples collected three months apart was rather low at 0.41 (95% CI 0.20-0.62). Neopterin is degraded by exposure to natural light and specimens must be collected and stored in light protected tubes. At the commencement of the pilot study, this was overlooked and several specimens were collected without light protection. However, it is natural, rather than artificial light that has been shown to breakdown neopterin and all samples were collected indoors in a blood-collecting centre.
Immediately after collection the samples were refrigerated and then stored in a \(-20^\circ C\) freezer, both of which are darkened.

If lack of light protection had indeed caused a breakdown of neopterin, the non-light protected specimens would be expected to have a lower level of neopterin than the light protected specimens. In particular, it seems likely that in comparison with their pair (from the same participant) we would expect the neopterin levels of all the non-light protected samples to be lower than their pairs. However, this was not the case. Figure 5.4 shows that the difference in neopterin levels between specimens from two different collection times, where one specimen was not light protected, was relatively evenly distributed around zero. This lack of a consistent direction to the difference between the samples of a pair suggests that the lack of light protection has made little difference to the neopterin levels of the specimens.

In addition, two samples were recorded by the laboratory as being haemolysed. In these two samples, there was a consistent positive difference between the haemolysed sample and the non-haemolysed sample. This may indicate release of cellular neopterin to the serum and these results were dropped from further analysis.
Figure 5.4. Difference in serum neopterin between pairs where one is not light protected.

Note: The x-axis is the difference in the serum neopterin level between the light protected sample and the non-light protected sample.

The intraclass correlation, excluding these two haemolysed samples (but including the non-light protected samples) was 0.39 (95% CI 0.17-0.6).

The neopterin results included in the main part of the analysis are the averaged neopterin level in each pair, except those in which one sample was haemolysed. For these participants, only the value of the non-haemolysed sample was used.

There is no significant variation in the difference between neopterin levels in the two samples, depending on the month of blood collection in the pilot study. However, there is statistically significant variation in serum neopterin levels by month of blood collection.
in the 2001 study, with lowest levels in late winter. Month of blood collection is included in subsequent models.

**Figure 5.5 Serum neopterin by month of blood collection.** (Bars are mean ±SE mean connected by a cubic spline smoother, calculated for the transformed, normalized variable, then converted to neopterin values)

---

**5.4.5 Salivary IgA**

The intraclass correlation for salivary IgA measured at two time points, three months apart is low, at 0.35 (95% CI 0.13-0.58). Salivary IgA samples are sensitive to repeated thawing and refreezing as saliva contains proteolytic enzymes that destroy the antibody. In the early stages of the analysis of the samples, seventeen samples (out of a total of 420) were subjected to thawing during sample preparation and dilution. Note was taken of these specimen numbers.
Examination was made of the difference in salivary IgA levels between samples, where one sample of a pair (from the same person at different times) had been thawed. If thawing and subsequent proteolysis had occurred, the thawed specimen would be expected to have a lower IgA concentration than the undamaged specimen. If thawing had no effect, we might expect some specimens to have lower IgA levels than their pairs and some to have higher (note: it was not always the first or the second sample which had been thawed, rather it was random).

In all of the samples in which one of the pair had been repeatedly thawed, the thawed sample had a lower IgA value than the non-thawed pair. The differences ranged from 41.1mg/ml to 1058.6 mg/ml. This suggests that the thawing process had indeed damaged these samples and the measured level was probably not accurate. When these pairs were omitted from the analysis, the ICC improved to 0.64 (95% CI 0.41-0.86).

The concentration of salivary IgA showed no seasonal variation in this study population and there is no difference in the change in IgA concentration depending on the month of initial or final saliva collection. Research indicates that salivary IgA concentration peaks on awakening, falls steeply over the next four hours, and then levels flatten out and remain relatively stable over the day [389]. Efforts were made to avoid this diurnal rhythm by not collecting samples early in the morning and recording the time of sample collection. In addition, all samples were collected two hours post-prandially to standardise collection in relation to food intake. In this study sample, there was no
significant variation in salivary IgA concentration related to time of collection of the sample, and no significant difference in concentration between samples collected before 10am and those collected after 10am.

For those participants from the pilot study where there were two valid IgA measurements, these are averaged and included in the main analysis. For those pilot participants where one specimen had been repeatedly thawed, only the IgA concentration of the unaffected specimen was included. In addition, there were five participants for whom both saliva specimens had undergone repeated thawing – these results were dropped from the IgA analysis.

The normal range for salivary IgA in non-fasting adults is 10-105 mg/L; fasting adults 20-500 mg/L, although this range was established using a slightly different ELISA technique. Several of the results in this study were much higher than these normal ranges, e.g. 2000 mg/L. It is possible that these participants were developing upper respiratory infections with consequent elevation of salivary IgA levels. Subsequent analyses were repeated after dropping extreme values (greater than two standard deviations from the mean) but with no alteration of the results.

5.5 Correlation between the biomarkers.

It might be expected that there would be a high correlation between these markers, all of which were chosen on the basis of their probable association with activation of the
neuroendocrine stress response. However, they are representatives of different body systems and it is clear that despite biological connections between such systems, covariation is not absolute.

A correlation matrix using Pearson correlation and transformed markers (Table 5.8, presented here by the marker name) indicates the markers are generally not significantly correlated.

Table 5.8. Correlation matrix for biomarkers – male in lower half; female in upper half (italics).

<table>
<thead>
<tr>
<th></th>
<th>Fibrinogen</th>
<th>Waist-hip ratio</th>
<th>Salivary IgA</th>
<th>Neopterin</th>
<th>Glycated haemoglobin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>1.000</td>
<td>0.39 p = 0.0001</td>
<td>0.046 p = 1.000</td>
<td>0.17 p = 1.000</td>
<td>0.29 p = 0.01</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>0.13 p = 1.000</td>
<td>1.000</td>
<td>-0.02 p = 1.000</td>
<td>0.04 p = 1.000</td>
<td>0.27 p = 0.02</td>
</tr>
<tr>
<td>Salivary IgA</td>
<td>0.02 p = 1.000</td>
<td>0.15 p = 0.34</td>
<td>1.000</td>
<td>0.12 p = 1.000</td>
<td>-0.09 p = 1.000</td>
</tr>
<tr>
<td>Neopterin</td>
<td>0.05 p = 1.000</td>
<td>0.03 p = 1.000</td>
<td>0.07 p = 1.000</td>
<td>1.000</td>
<td>-0.014 p = 1.000</td>
</tr>
<tr>
<td>Glycated haemoglobin</td>
<td>0.23 p = 0.04</td>
<td>-0.08 p = 1.000</td>
<td>0.12 p = 1.000</td>
<td>0.02 p = 1.000</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Bonferroni correction applied

5.6 Variation of biological markers

The remainder of this chapter focuses on the analysis of the variation in each biological marker. Bivariate analysis of each marker and the range of socioeconomic, behavioural, psychosocial, work and health variables is considered. The various measures of SES were grouped according to different facets of socioeconomic status. Thus measures of income
included personal income, total household income and calculated (adjusted for number of individuals in the household) equivalent income. Occupational measures included personal occupational level (derived from the ASCO II coding system) and derived personal occupational status and the highest occupational status of any individual in the household (household occupational status). A single variable measured the highest educational achievement attained by the participant (coded to the ASCED). The type of current accommodation and the total number of cars in the household were used as measures of wealth. Perceived SES consisted of perceived position in the community and perceived position in Australia. Composite SES refers to the variable derived from principal components analysis of all of the SES measures.

There are significant correlations between different SES measures, but each marker was tested separately against each facet of SES to examine the particular association of each to marker variability.

Following bivariate analysis of associations with SES, each marker was considered in relation to each of the psychosocial, behavioural, biological and health variables, grouped in the following way:

- Behavioural variables included alcohol intake, several measures of physical activity, smoking status and number of cigarettes smoked per week.
- Strain variables included measures of economic strain or financial hardship, perceived stress (two measures), household responsibility, and total number of recent life events.
• Psychosocial variables included measures of social capital, friendship and pet ownership, as well as the two summary personality variables (see Methods, section 4.12), two summary coping factors and measures of depression and anxiety.

• Childhood circumstances were measured by using a number of measures of parental SES when the participant was aged 12 years (education, occupation and occupational status), as well as the summary measure of childhood adversity.

• Biological variables included serum cholesterol, serum HDL cholesterol, serum LDL cholesterol, serum triglycerides, and serum fasting glucose. Health variables included self-rated health, weight and body mass index and status with respect to medications taken.

• Work variables included measures of job demands and control, as well as the individual elements of job control, skill discretion and decision authority, and a summary job strain variable (based on the Karasek model [467]). The statistical analysis also examined associations with the number of hours worked per week, amount of time off work in the last month, and employment status, as well as measures examining the level of job security, likelihood of being able to obtain similar level work if this job was lost and the extent to which the participant felt that the rewards associated with the job were fair.

Although the problem of multiple comparisons is recognised when such a large number of bivariate analyses are conducted, no Bonferroni correction was applied. This means that some of the apparently statistically significant results may be spurious and simply
due to chance. However, these analyses were performed only to guide the model building process and to explore possible associations within the data.

Following the bivariate analyses, mediation analysis was used to examine the third hypothesis, i.e. psychosocial factors mediate the association between measures of SES and the biological markers. This analysis was carried out on variables grouped as above – behavioural and psychosocial variables, childhood circumstances, health/biological variables, and work variables, to examine which of the elements within these groups had the most important mediating role in the relationship between measures of SES and the markers. While it is recognized that several of the markers had no statistically significant association with several of the SES measures, it is possible to have positive and negative mediating effects from intermediate variables, that ‘cancel each other out’ so that the net effect is non-significant, even though significant mediation is occurring [489]. In view of the problems outlined in section 4.14.1, I have presented significant mediating effects rather than the proportion of total effect mediated.

Finally multiple regression models were constructed to examine both the interplay of socioeconomic measures with behavioural, psychosocial, work and health measures and the relative strength of the independent associations of each of these variables, with each marker (by comparing standardised beta coefficients as variables are measured in a range of different units).
This was undertaken in several steps. First a model was created using the marker as the dependent variable and measures of socioeconomic status as the explanatory variables. Since the determinants of the markers seemed to be quite different for men and women, the analyses were conducted separately by gender. All indices of SES were entered in the model, and then removed in order of the highest on ‘p’ values, checking after each removal with likelihood ratios as to whether the removal caused a significant change in the model. Once the final model of the marker and measures of SES was developed, the remaining explanatory variables were entered in groups – behavioural variables, psychosocial variables, biological measures, work variables etc. With each group of explanatory variables, those with p values of greater than 0.2 were removed from the model, using likelihood ratios to ensure the model suffered no significant loss of explanatory power. When all variables had been entered into the model, a final model was developed which explained the maximum variance using the least number of explanatory variables. Following this, variables identified by literature review as being important to include in the model were re-entered and the model fit re-examined repeatedly. The SES variables from the original model were retained in order to examine the strength of their independent effects, as were possible confounders identified from the literature or suggested by the bivariate analyses.

5.6.1 Fibrinogen

Fibrinogen is measured in grams/litre with a normal range of 1.5-4.0 g/L. The median (range) in the 2001 sample was 2.3 (1.1-4.7) g/L.
Log transformation was used to normalize the skewed distribution of values of plasma fibrinogen, (skewness = 1.10, transformed skewness = 0.18).

**Figure 5.6. Transformation of plasma fibrinogen**

_Variation with SES_

There were statistically significant correlations only between log plasma fibrinogen and number of cars in the household for males, and occupational SES measures for females. However, at least in women, there appears to be a trend to a negative association between SES and plasma fibrinogen for almost all SES variables, i.e. plasma fibrinogen tends to be lower in those with higher SES. Childhood SES, as measured by father’s occupational status and the composite SES measure also show statistically significant associations with plasma fibrinogen level of female participants.

These bivariate analyses indicate likely gender differences in the socioeconomic determinants of plasma fibrinogen in this sample, so that the remainder of the analysis was performed with groups separated by gender. Similar trends were seen between SES variables and plasma fibrinogen levels in the pilot data, but these were not significant.
Due to the small sample size of the pilot study, no further analyses were performed on those data.

Table 5.9. Correlation of log fibrinogen with different measures of socioeconomic status

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th></th>
<th>Females</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Personal income</td>
<td>-0.01</td>
<td>0.87</td>
<td>-0.07</td>
<td>0.47</td>
</tr>
<tr>
<td>Household income</td>
<td>-0.08</td>
<td>0.32</td>
<td>-0.15</td>
<td>0.09</td>
</tr>
<tr>
<td>Equivalent household income</td>
<td>0.03</td>
<td>0.69</td>
<td>-0.17</td>
<td>0.05</td>
</tr>
<tr>
<td>Occupational level</td>
<td>0.05</td>
<td>0.56</td>
<td>-0.18</td>
<td>0.04</td>
</tr>
<tr>
<td>Personal occupational status</td>
<td>0.04</td>
<td>0.64</td>
<td>-0.23</td>
<td>0.009</td>
</tr>
<tr>
<td>Household occupational status</td>
<td>-0.01</td>
<td>0.90</td>
<td>-0.22</td>
<td>0.02</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>-0.01</td>
<td>0.90</td>
<td>-0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>Accommodation type</td>
<td>-0.06</td>
<td>0.48</td>
<td>-0.08</td>
<td>0.36</td>
</tr>
<tr>
<td>Number of cars in household</td>
<td>-0.26</td>
<td>0.001</td>
<td>-0.02</td>
<td>0.84</td>
</tr>
<tr>
<td>Perceived position in community</td>
<td>-0.02</td>
<td>0.76</td>
<td>-0.003</td>
<td>0.97</td>
</tr>
<tr>
<td>Perceived position in Australia</td>
<td>-0.03</td>
<td>0.75</td>
<td>-0.07</td>
<td>0.41</td>
</tr>
<tr>
<td>Mother’s educational level</td>
<td>-0.10</td>
<td>0.21</td>
<td>-0.05</td>
<td>0.60</td>
</tr>
<tr>
<td>Father’s educational level</td>
<td>0.008</td>
<td>0.92</td>
<td>-0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>Father’s occupational level</td>
<td>-0.06</td>
<td>0.44</td>
<td>-0.12</td>
<td>0.18</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>-0.04</td>
<td>0.65</td>
<td>-0.21</td>
<td>0.009</td>
</tr>
<tr>
<td>Childhood household occupational status</td>
<td>-0.08</td>
<td>0.32</td>
<td>-0.27</td>
<td>0.004</td>
</tr>
<tr>
<td>Composite SES</td>
<td>-0.08</td>
<td>0.30</td>
<td>-0.26</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Note: all SES measures have been recoded so that higher values denote higher SES.
**Variation with other measures**

**Behavioural risk factors:** Increasing frequency of alcohol intake had a statistically significant association with decreasing levels of plasma fibrinogen in men and women (p = 0.03, p = 0.005 respectively). Number of cigarettes per week had a weakly statistically significant positive association with plasma fibrinogen in men (p = 0.04). While there was no statistically significant association between any level of physical activity and plasma fibrinogen in this sample, the negative association with vigorous activity was of borderline significance in males (p = 0.05).

**Strain variables:** Economic strain, particularly in females, was positively associated with plasma fibrinogen (p = 0.04, p = 0.02 for two different economic strain measures). However, there was no statistically significant association between plasma fibrinogen and any measure of perceived stress, life events or household responsibility in either gender.

**Psychosocial variables:** There was a negative, statistically significant association between negative coping and plasma fibrinogen in women (r = -0.22, p = 0.01), but no other statistically significant associations with any personality measure, social capital, social support, depression or anxiety.

**Biological and health variables:** Weight and body mass index in women had positive and statistically significant associations with plasma fibrinogen (p = <0.0001 in both cases). In men, increased body mass index and lower levels of HDL cholesterol were
associated with higher levels of plasma fibrinogen ($p = -0.02$, $p = 0.004$ respectively). There were no statistically significant associations between plasma fibrinogen and other biological measures or self-rated health, and the positive association between diastolic blood pressure and plasma fibrinogen just failed to reach statistical significance ($p = 0.05$).

**Work factors**: In men there was a positive, statistically significant association between plasma fibrinogen and skill discretion ($p < 0.001$), but no statistically significant association between other measures of job control, work demands or job strain and plasma fibrinogen in either sex. Sense of job fairness had a statistically significant positive association with plasma fibrinogen in women.

In bivariate analysis, the correlates of plasma fibrinogen levels in this study appear similar to those described elsewhere (see section 3.8). Smoking, lack of exercise, elevated diastolic blood pressure, decreased serum HDL cholesterol and increased body mass index and weight are common risk factors associated with both elevated levels of fibrinogen and cardiovascular disease. Other associations, such as those with measures of work conditions have been found by others [78] and implicated causally with increased risk of cardiovascular disease. The positive association between levels of plasma fibrinogen and measures of perceived economic strain has not been reported elsewhere to my knowledge.
Mediation analysis

Tables 5.10 to 5.19 summarise the results of mediation analysis examining the association between plasma fibrinogen levels and elements of SES. Although each measure of SES was analysed separately, and there were non-significant bivariate correlations with most SES measures, the mediators of different elements of SES are very similar. Thus job control, number of hours worked per week, perceived economic strain, smoking, physical activity, alcohol intake, use of active coping styles and perceived stress are common threads throughout this analysis. In addition father’s occupational status contributes significantly to the association between plasma fibrinogen and SES.

Income:

Table 5.10. Statistically significant mediators of the association between plasma fibrinogen levels and personal income.

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>p&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Moderate physical activity</td>
<td>ns</td>
<td>p = 0.03</td>
</tr>
<tr>
<td>Frequency of alcohol intake</td>
<td>ns</td>
<td>p = 0.01</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Household responsibility</td>
<td>p = 0.022</td>
<td>ns</td>
</tr>
<tr>
<td>Perceived stress</td>
<td>p = 0.018</td>
<td>ns</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.008</td>
<td>ns</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>p = 0.024</td>
<td>ns</td>
</tr>
<tr>
<td>Job control</td>
<td>p &lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Hours worked per week</td>
<td>p &lt;0.001</td>
<td>p &lt;0.001</td>
</tr>
<tr>
<td>Skill discretion</td>
<td>ns</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Decision authority</td>
<td>p = 0.005</td>
<td>ns</td>
</tr>
<tr>
<td>Positive interaction with friends and family</td>
<td>ns</td>
<td>p = 0.024</td>
</tr>
</tbody>
</table>
Household income:

Table 5.11. Statistically significant mediators of the association between plasma fibrinogen levels and household income.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>p&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Frequency of alcohol intake</td>
<td>p = 0.01</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p&lt;0.001</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.010</td>
<td>ns</td>
</tr>
<tr>
<td>Sense of belonging and trust in others</td>
<td>p = 0.009</td>
<td>ns</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>p = 0.033</td>
<td>ns</td>
</tr>
<tr>
<td>Job control</td>
<td>p&lt;0.001</td>
<td>p = 0.010</td>
</tr>
<tr>
<td>Hours worked per week</td>
<td>p = 0.004</td>
<td>p = 0.012</td>
</tr>
<tr>
<td>Decision authority</td>
<td>p = 0.002</td>
<td>ns</td>
</tr>
<tr>
<td>High job control, high work demands</td>
<td>p&lt;0.001</td>
<td>p = 0.032</td>
</tr>
<tr>
<td>Positive interaction with friends and family</td>
<td>p = 0.023</td>
<td>ns</td>
</tr>
</tbody>
</table>

Equivalent income:

Table 5.12. Statistically significant mediators of the association between plasma fibrinogen levels and household equivalent income.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of alcohol intake</td>
<td>p = 0.014</td>
<td>p = 0.014</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p&lt;0.001</td>
<td>p = 0.005</td>
</tr>
<tr>
<td>Household responsibility</td>
<td>ns</td>
<td>p = 0.005</td>
</tr>
<tr>
<td>Perceived stress</td>
<td>p = 0.040</td>
<td>ns</td>
</tr>
<tr>
<td>Decision authority</td>
<td>ns</td>
<td>p = 0.018</td>
</tr>
<tr>
<td>Job control</td>
<td>p = 0.013</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Sense of job fairness</td>
<td>ns</td>
<td>p = 0.007</td>
</tr>
<tr>
<td>Number of hours worked per week</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>High job control, high work demands</td>
<td>p = 0.027</td>
<td>p = 0.044</td>
</tr>
<tr>
<td>Glycated haemoglobin</td>
<td>ns</td>
<td>p = 0.029</td>
</tr>
</tbody>
</table>
### Occupational level:

Table 5.13. Statistically significant mediators of the association between plasma fibrinogen levels and personal occupational level.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
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</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>p = 0.003</td>
<td>ns</td>
</tr>
<tr>
<td>Frequency of alcohol intake</td>
<td>ns</td>
<td>p = 0.043</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p&lt;0.001</td>
<td>p = 0.015</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.004</td>
<td>ns</td>
</tr>
<tr>
<td>Social capital 1</td>
<td>ns</td>
<td>p = 0.045</td>
</tr>
<tr>
<td>Highest parental occupational status</td>
<td>p = 0.004</td>
<td>p = 0.019</td>
</tr>
<tr>
<td>Skill discretion</td>
<td>p&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Job control</td>
<td>p &lt;0.001</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Number of hours worked per week</td>
<td>ns</td>
<td>p = 0.026</td>
</tr>
<tr>
<td>High job control, high work demands</td>
<td>p = 0.048</td>
<td>p = 0.012</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>ns</td>
<td>p = 0.035</td>
</tr>
<tr>
<td>Glycated haemoglobin</td>
<td>ns</td>
<td>p = 0.012</td>
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</table>

### Occupational status:

Table 5.14. Statistically significant mediators of the association between plasma fibrinogen levels and personal occupational status.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>p&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p&lt;0.001</td>
<td>p = 0.010</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.004</td>
<td>ns</td>
</tr>
<tr>
<td>Sense of belonging and trust in others</td>
<td>ns</td>
<td>p = 0.026</td>
</tr>
<tr>
<td>Highest parental occupational status</td>
<td>p = 0.002</td>
<td>p = 0.020</td>
</tr>
<tr>
<td>Father’s education</td>
<td>p = 0.010</td>
<td>ns</td>
</tr>
<tr>
<td>Mother’s education</td>
<td>ns</td>
<td>p = 0.028</td>
</tr>
<tr>
<td>Work demands</td>
<td>ns</td>
<td>p = 0.011</td>
</tr>
<tr>
<td>Job control</td>
<td>p &lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Number of hours worked per week</td>
<td>ns</td>
<td>p = 0.027</td>
</tr>
<tr>
<td>High job control, high work demands</td>
<td>p = 0.048</td>
<td>p = 0.003</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>ns</td>
<td>p = 0.009</td>
</tr>
<tr>
<td>Glycated haemoglobin</td>
<td>ns</td>
<td>p = 0.021</td>
</tr>
<tr>
<td>Body mass index</td>
<td>ns</td>
<td>p = 0.006</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>p = 0.010</td>
<td>ns</td>
</tr>
</tbody>
</table>
Household occupational status:

Table 5.15. Statistically significant mediators of the association between plasma fibrinogen levels and household occupational status.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>p&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p&lt;0.001</td>
<td>p = 0.003</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.016</td>
<td>ns</td>
</tr>
<tr>
<td>Sense of belonging and trust in others</td>
<td>ns</td>
<td>p = 0.026</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>p = 0.004</td>
<td>p = 0.014</td>
</tr>
<tr>
<td>Father’s education</td>
<td>p = 0.012</td>
<td>ns</td>
</tr>
<tr>
<td>Mother’s education</td>
<td>ns</td>
<td>p = 0.021</td>
</tr>
<tr>
<td>Work demands</td>
<td>ns</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>Job control</td>
<td>p = 0.003</td>
<td>p = 0.005</td>
</tr>
<tr>
<td>Skill discretion</td>
<td>P&lt;0.001</td>
<td>ns</td>
</tr>
<tr>
<td>High job control, high work demands</td>
<td>ns</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>Positive interaction with friends and family</td>
<td>p = 0.023</td>
<td>ns</td>
</tr>
<tr>
<td>Body mass index</td>
<td>ns</td>
<td>p = 0.005</td>
</tr>
<tr>
<td>Waist-hip ratio</td>
<td>p = 0.037</td>
<td>ns</td>
</tr>
</tbody>
</table>

Educational level:

Table 5.16. Statistically significant mediators of the association between plasma fibrinogen levels and highest attained education of participant.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest parental occupational status</td>
<td>p = 0.037</td>
<td>ns</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>p = 0.030</td>
<td>ns</td>
</tr>
<tr>
<td>Time set aside for relaxation</td>
<td>ns</td>
<td>p = 0.025</td>
</tr>
</tbody>
</table>

Accommodation type: None of the measured variables provided significant mediation of the association between accommodation type and plasma fibrinogen.
**Number of cars in the household:** Only economic strain had a significant mediating effect in the association between plasma fibrinogen and number of cars in the household, and only in women.

**Community ladder:**

Table 5.17. Statistically significant mediators of the association between plasma fibrinogen levels and perceived position in the community.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Economic strain</td>
<td>p = 0.021</td>
<td>p = 0.033</td>
</tr>
<tr>
<td>Total serious life events</td>
<td>p = 0.002</td>
<td>ns</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.005</td>
<td>ns</td>
</tr>
<tr>
<td>Sense of belonging and trust in others</td>
<td>p = 0.008</td>
<td>p = 0.046</td>
</tr>
<tr>
<td>Depression</td>
<td>p = 0.020</td>
<td>ns</td>
</tr>
<tr>
<td>Anxiety</td>
<td>p = 0.009</td>
<td>ns</td>
</tr>
<tr>
<td>Positive personality (high self-esteem, optimism etc)</td>
<td>p = 0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Positive interaction with family and friends</td>
<td>ns</td>
<td>p = 0.044</td>
</tr>
<tr>
<td>Decision authority</td>
<td>p = 0.007</td>
<td>ns</td>
</tr>
<tr>
<td>Job control</td>
<td>p = 0.001</td>
<td>p = 0.006</td>
</tr>
</tbody>
</table>

Table 5.18. Statistically significant mediators of the association between plasma fibrinogen levels and perceived position in Australia.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking status</td>
<td>p = 0.009</td>
<td>ns</td>
</tr>
<tr>
<td>Economic strain</td>
<td>p &lt; 0.001</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Active coping</td>
<td>p = 0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Social capital - trust</td>
<td>ns</td>
<td>p = 0.008</td>
</tr>
<tr>
<td>Sense of belonging and trust in others</td>
<td>p = 0.025</td>
<td>p = 0.011</td>
</tr>
<tr>
<td>Depression</td>
<td>p = 0.016</td>
<td>ns</td>
</tr>
<tr>
<td>Positive interaction with family and friends</td>
<td>p = 0.059</td>
<td>p = 0.017</td>
</tr>
<tr>
<td>Decision authority</td>
<td>p = 0.001</td>
<td>ns</td>
</tr>
<tr>
<td>Number of hours worked per week</td>
<td>p = 0.001</td>
<td></td>
</tr>
<tr>
<td>Job control</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Combination of high job control and high work demands</td>
<td>p = 0.026</td>
<td>p = 0.002</td>
</tr>
<tr>
<td>Fasting serum glucose</td>
<td>ns</td>
<td>p = 0.030</td>
</tr>
</tbody>
</table>
Table 5.19a Summary of significant mediation between SES and plasma fibrinogen (X p<0.001)

<table>
<thead>
<tr>
<th>Mediating variables</th>
<th>Personal income</th>
<th>Personal occupational level</th>
<th>Personal occupational status</th>
<th>Personal educational level</th>
<th>Household income</th>
<th>Household equivalent income</th>
<th>Household occupational status</th>
<th>Community position</th>
<th>Position in Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current smoking</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Moderate phys. activity</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Freq alcohol intake</td>
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<td></td>
<td>X</td>
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<tr>
<td>Economic strain</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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</tr>
<tr>
<td>Household responsibility</td>
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<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<tr>
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<td>X</td>
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<tr>
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Table 5.19b Summary of significant mediation between SES and plasma fibrinogen

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</table>
Tables 5.19a and b suggest that mediation by psychosocial factors is more important for men than women. Smoking, economic strain and work factors are most important in men, and work factors in women. In men, mediation occurs for both individual and household measures of SES, while for women the few significant mediation effects occur principally in household measures of SES.

**Multiple regression analysis**

In males, the simple model using only SES variables consisted only of the dependent variable, log fibrinogen, and number of cars in the household as the explanatory variable. The full model also contained active coping style, frequency of physical activity, smoking status, work demands, skill discretion, number of hours worked per week, sense of job fairness, diastolic blood pressure and HDL cholesterol. This model explained 36% of the variation in log fibrinogen (p<0.0001). The largest standardised beta coefficient was for having any cars, compared to no car (one car, beta = -0.70, two cars, beta = -0.86). However, only two male participants had no car, so this result may be misleading. Vigorous physical activity (beta = -0.40), skill discretion (beta = 0.36), depression (beta = 0.30), anxiety (beta = -0.29), diastolic blood pressure (beta = 0.27), HDL cholesterol (beta = -0.25) and being a smoker (beta = -0.20), also had important independent effects on plasma fibrinogen levels in men. Note that although there was no statistically significant association between plasma fibrinogen and depression or anxiety in univariate analysis, these variables have strong effects in the regression model, but in opposite directions. Although depression and anxiety should be distinct, the Goldberg scale may
not be distinguishing clearly between them and the equal magnitude but opposite sign suggests that in this analysis, they are collinear.

In females, the simple model included log fibrinogen as the dependent variable and income, household status and equivalent income as the SES explanatory variables. The full model included frequency of alcohol intake, job security, father’s occupational level, ownership of a pet, body mass index, serum cholesterol, HDL cholesterol, serum triglycerides, LDL cholesterol and marital status (specifically never having been married, compared to currently being married). This model accounted for 43.8% of the variation in plasma fibrinogen (p<0.0001). The strongest independent associations with plasma fibrinogen were for total cholesterol (beta = -4.25), LDL cholesterol (beta = 3.48), HDL cholesterol (beta = -1.63), triglycerides (beta = 1.45), and BMI (beta = -0.75). Of the SES variables, household status (beta = -0.29) had the strongest association with plasma fibrinogen, but this was relatively weak compared to the association with serum lipids and body mass index.

5.6.2 Glycated haemoglobin

Glycated haemoglobin (HbA1c) is measured as a percent of haemoglobin that is glycated, with a normal range of 4.5-6.0 %. The median (range) in the 2001 study sample was 4.6% (3.6% - 5.9 %). In the 2001 study population there was a non-normal distribution (skewness = 0.26). Transformation by taking the natural log resulted in a relatively normal distribution (skewness = -0.03).
Figure 5.7 Transformation of glycated haemoglobin

![Graph showing transformation of glycated haemoglobin.]

### Variation with SES

Table 5.20. Variation of glycated haemoglobin with measures of SES

<table>
<thead>
<tr>
<th>Measure</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
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<td>Personal income</td>
<td>-0.05 0.55</td>
<td>-0.10 0.27</td>
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<tr>
<td>Household income</td>
<td>-0.11 0.18</td>
<td>-0.15 0.09</td>
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<tr>
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<td>0.03 0.75</td>
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<td>-0.24 0.008</td>
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<td>-0.24 0.008</td>
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<td>-0.19 0.03</td>
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<td>Accommodation type</td>
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<td>-0.03 0.74</td>
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<tr>
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<td>-0.14 0.09</td>
<td>0.02 0.86</td>
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<tr>
<td>Perceived position in community</td>
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<td>Perceived position in Australia</td>
<td>-0.18 0.03</td>
<td>-0.04 0.68</td>
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<td>-0.13 0.12</td>
<td>-0.05 0.61</td>
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<tr>
<td>Father’s educational level</td>
<td>-0.03 0.69</td>
<td>-0.02 0.82</td>
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<td>-0.13 0.15</td>
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<tr>
<td>Childhood household occupational status</td>
<td>-0.16 0.06</td>
<td>-0.12 0.20</td>
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<tr>
<td>Composite SES</td>
<td>-0.11 0.17</td>
<td>-0.23 0.01</td>
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</table>

For men, the only statistically significant association between levels of glycated haemoglobin and any measure of SES, was with perceived position in Australia, although the general trend was to a negative association (lower levels of glycated haemoglobin...
with higher SES). Due to the number of comparisons this single significant association may be due to chance rather than a genuine association.

In women, there were statistically significant associations with equivalent household income, personal occupational measures and education. Most SES measures showed a negative association with glycated haemoglobin. Elevated levels of glycated haemoglobin are a reflection of past elevations of serum glucose and lack of control of blood glucose levels. While none of the current participants had abnormal levels of glycated haemoglobin, lower SES tends to be associated with less favourable levels of glycated haemoglobin.

**Variation with other measures**

**Behavioural variables:** There were no statistically significant associations between glycated haemoglobin levels and alcohol intake, or mild, moderate or vigorous physical activity. For males only, current smokers had significantly higher levels of glycated haemoglobin than did non-smokers (p = 0.01) and there was a positive and significant association with the number of cigarettes smoked per week (p = 0.007).

**Strain variables:** Economic strain in males and financial hardship in females were associated with higher levels of glycated haemoglobin (p = 0.03, p = 0.03 respectively). However, there was no statistically significant association of glycated haemoglobin levels with any measure of self-rated stress, household responsibility or number of serious recent life events.
**Psychosocial variables:** In males, active coping showed a statistically significant negative association with levels of glycated haemoglobin in males (p = 0.0002) while depression and anxiety were both positively associated with levels of glycated haemoglobin in women (p = 0.04, p = 0.02 respectively). There was no association with measures of personality, or any measures of social capital or number of friends. Positive interaction with family and friends was associated with decreasing levels of glycated haemoglobin and negative interaction with increasing glycated haemoglobin in males (p = 0.02, p = 0.03 respectively).

**Biological variables:** As expected in view of its close association with serum glucose levels, there was a statistically significant positive association between levels of glycated haemoglobin and fasting serum glucose in males and females (p = 0.01, p = <0.0001 respectively). For women, but not men, there was a statistically significant positive association between glycated haemoglobin levels and weight (p = 0.02) and body mass index (p = 0.03). Serum lipids (total cholesterol, triglycerides, HDL and LDL cholesterol) were not statistically significantly associated with glycated haemoglobin in this study sample.

**Work variables:** Within the variables examining working conditions only measures of job control (skill discretion: males r = -0.24, p = 0.004, females r = -0.23, p = 0.01) and number of hours worked per week (r = -0.19, p = 0.02, males only) were significantly
associated with blood levels of glycated haemoglobin. In particular, the association with
work demands and the summary measures of job strain were not statistically significant.

**Mediation analysis**

Tables 5.21 and 5.22 summarize the results of the mediation analysis for glycated
haemoglobin.

**Table 5.21. Mediating variables in the association of glycated haemoglobin and SES.**

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<tr>
<th></th>
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<th>Females</th>
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None of the measured psychosocial or behavioural variables provided statistically
significant mediation of the association between glycated haemoglobin and any measure
of SES in females. For males, work variables, but particularly having an active coping
style, provided important mediation in this association.
Table 5.22. Summary of the significant mediation in the association between measures of SES and glycated haemoglobin

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<table>
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</tr>
</tbody>
</table>

**Multiple regression analysis**

The multiple regression model of SES and glycated haemoglobin for males, contained only perceived position in Australia (p = 0.01, Adj R² = 0.03). The full model contained mother’s education, child adversity, positive personality, financial problems, self-rated health, skill discretion, job security, number of hours worked per week, positive interaction with family or friends, salivary IgA, fasting glucose, weight and body mass index. This model accounted for 28% of the variation in glycated haemoglobin (p = 0.0001). The strongest associations with glycated haemoglobin in this model are with body mass index (beta = -0.57), weight (beta = 0.47), fasting blood glucose (beta = 0.26), positive social support (beta = -0.24), salivary IgA (beta = 0.22) and job security (beta = -
0.33 for “extremely secure” compared to “not at all secure”). In this model, perceived
position in Australia had a non-significant association with glycated haemoglobin (beta =
0.08, p = 0.43). (Body mass index and weight are collinear, but the model explains
significantly less of the variation in log glycated haemoglobin if either is excluded).

In women, the simple model included only occupational level. While bivariate
correlations suggested that equivalent income, education and occupational status had a
statistically significant association with glycated haemoglobin levels, a model containing
only occupation provided the best fit (p = 0.008, Adj R^2 = 0.05). A full model, testing all
biological, psychosocial and behavioural variables contained diastolic blood pressure,
waste-hip ratio, fasting glucose, negative coping style, anxiety and number of friends.
This model explained 27% of the variation in log HbA_1c (p<0.0001). Anxiety (beta =
0.34), fasting glucose (beta = 0.23), and WHR (beta = 0.20) showed the strongest
independent associations, but the association with occupational level remained
statistically significant (beta = 0.18, p = 0.04).

5.6.3 Waist-hip ratio

Waist-hip ratio (WHR) had an approximately normal distribution, and was therefore not
transformed. The “normal” range varies by age and sex, but in an Australian study of 40-
44 year old adults, the mean and standard deviation were: men 0.89 (SD 0.05); women
0.76 (SD 0.05). In the Biomarkers Study, the mean WHR in men was 0.90 (SD 0.06,
range 0.77-1.08) and in women 0.76 (SD 0.05, range 0.66-0.88).
Variation with SES

Table 5.23. Correlation of waist-hip ratio with measures of SES.

<table>
<thead>
<tr>
<th>Measure</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
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<td>Household income</td>
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<td>Equivalent household income</td>
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</tr>
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<td>0.0008</td>
</tr>
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<td>0.004</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>-0.24</td>
<td>0.002</td>
</tr>
<tr>
<td>Accommodation type</td>
<td>-0.03</td>
<td>0.66</td>
</tr>
<tr>
<td>Number of cars in household</td>
<td>0.04</td>
<td>0.58</td>
</tr>
<tr>
<td>Perceived position in community</td>
<td>-0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>Perceived position in Australia</td>
<td>-0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>Mother’s educational level</td>
<td>-0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>Father’s educational level</td>
<td>-0.18</td>
<td>0.02</td>
</tr>
<tr>
<td>Father’s occupational level</td>
<td>-0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>-0.22</td>
<td>0.004</td>
</tr>
<tr>
<td>Childhood household status</td>
<td>-0.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Composite SES</td>
<td>-0.27</td>
<td>0.0007</td>
</tr>
</tbody>
</table>

The associations between waist-hip ratio and socioeconomic status (Table 5.23) were stronger for males than females in this study sample. There were particularly strong associations with measures of occupational level and educational attainment and with measures of childhood socioeconomic status. The apparent association of WHR with perceived socioeconomic position could be influenced by reverse causation – those with high waist-hip ratio could view themselves less favourably because of their body shape and thus score themselves poorly on this measure. In general the trend is to higher waist-hip ratio with lower SES, on almost all measures of current and childhood SES.
**Variation with other measures**

**Behavioural variables:** Waist-hip ratio showed no statistically significant association with frequency of alcohol intake, smoking status, number of cigarettes per week or mild physical activity in men or women. However, moderate and vigorous physical activity were associated with decreased waist-hip ratio in men but not in women (p = 0.006, p = 0.001 for moderate and vigorous activity, respectively).

**Strain measures:** There were no statistically significant associations between any measure of economic strain, perceived stress or number of life events and waist-hip ratio.

**Psychosocial factors:** WHR was not statistically significantly associated with any personality variables, coping factors, depression, anxiety, social capital or social support.

**Childhood circumstances:** There was a weakly significant positive association between WHR and childhood adversity in women (p = 0.04).

**Biological and health variables:** WHR had negative, statistically significant associations with self-rated health (p<0.0001), and physical health (as assessed in the SF-12, p = 0.006), and positive, statistically significant associations with fasting glucose (p<0.0001) and systolic blood pressure (p = 0.0003) in men only. In addition, there were statistically significant positive associations between WHR and diastolic blood pressure (men: p = 0.0001, women: p = 0.006), serum cholesterol (men: p = 0.02, women: p = 0.0015), body mass index (men: p<0.0001, women: p<0.0001) and weight (p<0.0001, p<0.0001) in men.
and women. There was a negative, statistically significant association with HDL cholesterol (men: p<0.0001, women: p<0.0001). LDL cholesterol showed a statistically significant positive association with WHR in women only (p = 0.0001).

**Work-related variables**: There was no statistically significant association with employment status, job demands, decision authority, job security, job marketability, job fairness, total number of hours worked per week, the amount of relaxation time available or the summary variable of job control. However, the association between WHR and skill discretion was statistically significant for men (r = -0.21, p = 0.004) and women (r = -0.26, p = 0.002) as was the combination of low job control and high job demands in men (p = 0.01; higher WHR compared to those with high control/high demand jobs).

**Mediation analysis**

Tables 5.24 and 5.25 summarize the results of the mediation analysis for WHR and SES.

**Table 5.24 Mediating variables in the association between WHR and SES.**

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mediating</td>
<td>Mediating</td>
</tr>
<tr>
<td></td>
<td>variable</td>
<td>variable</td>
</tr>
<tr>
<td>Personal income</td>
<td>None</td>
<td>Skill discretion 0.071</td>
</tr>
<tr>
<td>Household income</td>
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<td>Skill discretion 0.075</td>
</tr>
<tr>
<td>Equivalent household income</td>
<td>None</td>
<td>Skill discretion 0.058</td>
</tr>
<tr>
<td>Occupational level</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Personal occupational status</td>
<td>None</td>
<td>Body mass index 0.027</td>
</tr>
<tr>
<td>Household occupational status</td>
<td>Job marketability 0.064</td>
<td>Body mass index 0.038</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Accommodation type</td>
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<td>None</td>
</tr>
<tr>
<td>Number of cars in household</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Perceived position in community</td>
<td>Depression 0.022</td>
<td>None</td>
</tr>
<tr>
<td>Perceived position in Australia</td>
<td>Anxiety 0.042</td>
<td>Social capital 0.062</td>
</tr>
<tr>
<td></td>
<td>Depression 0.02</td>
<td>Active coping style 0.07</td>
</tr>
</tbody>
</table>
Although there appears to be a strong negative association between WHR and SES, there was little evidence of significant mediation by any of the measured behavioural or psychosocial variables. WHR and BMI are strongly correlated ($r = 0.46, p<0.0001$) and may be subject to similar causal influences, rather than BMI being on the causal pathway between WHR and SES. BMI is a measure of body fatness, whereas WHR is a measure of fat distribution. Other influences may operate to control the site of deposition of increasing body mass, producing variations in WHR. However, although the review of physiology suggests that psychosocial influences may guide the site of fat deposition, there is no suggestion of important mediation by these factors in this study. There is no evidence of mediation by any psychosocial variable in the association between BMI and WHR.

Table 5.25. Summary of significant mediation between SES and WHR

<table>
<thead>
<tr>
<th>Males Mediating variables</th>
<th>Personal income</th>
<th>Personal occupational level</th>
<th>Personal occupational status</th>
<th>Personal educational level</th>
<th>Household income</th>
<th>Household equivalent income</th>
<th>Household occupational status</th>
<th>Community position</th>
<th>Position in Australia</th>
</tr>
</thead>
<tbody>
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<tr>
<td>Depression</td>
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<td></td>
<td></td>
<td>X</td>
<td>X</td>
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<tr>
<td>Anxiety</td>
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<td>BMI</td>
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<td></td>
</tr>
<tr>
<td>Females</td>
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<td></td>
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<td></td>
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<tr>
<td>Depression</td>
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<td>Anxiety</td>
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<td></td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
Multiple regression analysis

Males: in the simple model, only personal occupational status remained in the model (p = 0.0001, Adj R$^2$ = 0.07). The full model, including the SES term consisted of body mass index, self rated health (good compared to excellent), job marketability (with a trend from not at all difficult to extremely difficult), active coping, diastolic blood pressure and job strain (high control, low demands) (p<0.0001, Adj R$^2$ = 0.57). The strongest associations were with BMI (beta = 0.51), personal occupational status (beta = -0.29), self rated health (beta = 0.25, for good compared to excellent health), diastolic blood pressure (beta = 0.18), and active coping (beta = -0.11). Those in high control/low demand work had significantly lower WHR (beta = -0.17) than any other combination of demands and control (and there were no significant differences between the other combinations). BMI alone explained much of the variation in WHR (p<0.0001, Adj R$^2$ = 0.397 in the model containing only WHR and BMI).

Females: In the simple model, only occupational level was explanatory for the variation in WHR (p = 0.012, Adj R$^2$ = 0.032). The full model included BMI, serum cholesterol, and serum HDL cholesterol in addition to occupational level (p<0.0001, Adj R$^2$ 0.38). On its own, BMI accounted for 24% of the variation in WHR. In the final model BMI had the strongest association with WHR (beta = 0.42), while total cholesterol (beta = 0.25) and HDL cholesterol (beta = -0.22) had associations of similar strength, but opposite direction.
5.6.4 Neopterin

The normal reference range for serum neopterin is adults is 5.4 ± 2.3 nmol/L. The median (range) for serum neopterin in this study sample was 5.5 (2.9-15.4) nmol/L. 9.7% of values were greater than the 10nmol/L, which is similar to that found by Maes in a population sample of normal volunteers [357].

Neopterin had a non-normal distribution (skewness = 2.78), which was transformed to a near-normal distribution using the reciprocal of the square root of neopterin (skewness = -0.84).

**Figure 5.8 Transformation of serum neopterin**

![Graph showing the transformation of serum neopterin](image)

**Variation with SES**

There was a general trend towards lower serum neopterin with higher SES. Effects appear to be stronger in women than in men, with the strongest associations for measures of current occupational status. Childhood socioeconomic situation and current income measures correlate poorly with neopterin levels in males and females.
Table 5.26. Correlation between serum neopterin and socioeconomic status.

<table>
<thead>
<tr>
<th></th>
<th>Males r</th>
<th>Males p</th>
<th>Females r</th>
<th>Females p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal income</td>
<td>-0.05</td>
<td>0.49</td>
<td>-0.10</td>
<td>0.24</td>
</tr>
<tr>
<td>Household income</td>
<td>-0.06</td>
<td>0.40</td>
<td>-0.09</td>
<td>0.24</td>
</tr>
<tr>
<td>Equivalent household income</td>
<td>-0.05</td>
<td>0.47</td>
<td>-0.12</td>
<td>0.14</td>
</tr>
<tr>
<td>Occupational level</td>
<td>-0.14</td>
<td>0.07</td>
<td>-0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>Personal occupational status</td>
<td>-0.02</td>
<td>0.82</td>
<td>-0.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Household occupational status</td>
<td>0.008</td>
<td>0.92</td>
<td>-0.24</td>
<td>0.003</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>-0.04</td>
<td>0.57</td>
<td>-0.005</td>
<td>0.95</td>
</tr>
<tr>
<td>Accommodation type</td>
<td>-0.04</td>
<td>0.60</td>
<td>0.08</td>
<td>0.35</td>
</tr>
<tr>
<td>Number of cars in household</td>
<td>0.10</td>
<td>0.16</td>
<td>-0.04</td>
<td>0.61</td>
</tr>
<tr>
<td>Perceived position in community</td>
<td>0.04</td>
<td>0.59</td>
<td>-0.17</td>
<td>0.06</td>
</tr>
<tr>
<td>Perceived position in Australia</td>
<td>-0.05</td>
<td>0.57</td>
<td>-0.14</td>
<td>0.13</td>
</tr>
<tr>
<td>Mother’s educational level</td>
<td>0.07</td>
<td>0.33</td>
<td>-0.06</td>
<td>0.46</td>
</tr>
<tr>
<td>Father’s educational level</td>
<td>0.03</td>
<td>0.77</td>
<td>-0.13</td>
<td>0.12</td>
</tr>
<tr>
<td>Father’s occupational level</td>
<td>-0.06</td>
<td>0.47</td>
<td>-0.09</td>
<td>0.31</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>0.01</td>
<td>0.88</td>
<td>-0.11</td>
<td>0.18</td>
</tr>
<tr>
<td>Childhood household occupational status</td>
<td>0.02</td>
<td>0.80</td>
<td>-0.07</td>
<td>0.39</td>
</tr>
<tr>
<td>Composite SES</td>
<td>-0.04</td>
<td>0.56</td>
<td>-0.21</td>
<td>0.009</td>
</tr>
</tbody>
</table>

**Variation with other measures**

**Behavioural variables:** No behavioural variables showed a statistically significant association with serum neopterin.

**Strain measures:** There were marginally significant positive associations with economic strain in females (p = 0.03, p = 0.06) for two different measures of strain (increasing strain associated with increasing serum neopterin). However, serum neopterin levels were not statistically significantly associated with any measure of perceived stress, household responsibility or recent life events in this study sample.
Psychosocial variables: Serum neopterin had a negative, statistically significant association with levels of trust and a sense of belonging (r = 0.22, p = 0.006) in men, but was not statistically significantly associated with depression, anxiety, social support, marital status, number of friends or personality style, in this study sample.

Biological variables: None of the measured biological variables had a statistically significant association with serum neopterin levels in males, apart from a negative association with HDL cholesterol (r = 0.16, p = 0.03). For women, serum neopterin had a negative and weakly statistically significant association with HDL cholesterol (r = 0.16, p = 0.04), but a stronger positive association with weight (r = 0.23, p = 0.004) and body mass index (r = 0.26, p = 0.003).

Work-related variables: There were no statistically significant associations with any work variable for men or women, apart from a positive association with job strain (low control/high demands, p = 0.002; low control/low demands, p = 0.03 compared to high control/high demands) for women only.

Mediation analysis

Even though the associations between SES and neopterin levels in bivariate analysis were weak, in mediation analysis there was a consistent pattern in which economic strain and number of cigarettes smoked per week were statistically significant mediators of the association between serum neopterin and SES in men. Economic strain was a positive mediator of this association, while the number of cigarettes smoked per week was a
negative mediator. Positive mediators are the route through which the explanatory variable has its effect on the dependent variable. On the other hand, negative mediators undermine this effect, so that increased cigarette smoking lessens the impact of SES on serum neopterin. Tables 5.27 and 5.28 summarize the results of the mediation analysis for serum neopterin.

Table 5.27. Mediating variables in the association between serum neopterin and SES.

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mediating variable</td>
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</tr>
<tr>
<td>Personal income</td>
<td>Economic strain</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>Low control, low</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>demand work</td>
<td></td>
</tr>
<tr>
<td>Household income</td>
<td>Economic strain</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>worked per week</td>
<td>0.010</td>
</tr>
<tr>
<td>Equivalent household</td>
<td>Economic strain</td>
<td>0.012</td>
</tr>
<tr>
<td>income</td>
<td>worked per week</td>
<td>0.010</td>
</tr>
<tr>
<td>Occupational level</td>
<td>Economic strain</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Cigarettes smoked per</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>week</td>
<td></td>
</tr>
<tr>
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<td>Economic strain</td>
<td>0.011</td>
</tr>
<tr>
<td>status</td>
<td>Cigarettes smoked per</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>week</td>
<td></td>
</tr>
<tr>
<td>Household occupational</td>
<td>Economic strain</td>
<td>0.011</td>
</tr>
<tr>
<td>status</td>
<td>Cigarettes smoked per</td>
<td>0.050</td>
</tr>
<tr>
<td></td>
<td>week</td>
<td></td>
</tr>
<tr>
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<td>None</td>
</tr>
<tr>
<td>Accommodation type</td>
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<td>None</td>
</tr>
<tr>
<td>Number of cars in</td>
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<td>None</td>
</tr>
<tr>
<td>household</td>
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<td></td>
</tr>
<tr>
<td>Perceived position in</td>
<td>Economic strain</td>
<td>0.038</td>
</tr>
<tr>
<td>community</td>
<td>Social capital</td>
<td>0.039</td>
</tr>
<tr>
<td>Perceived position in</td>
<td>Economic strain</td>
<td>0.006</td>
</tr>
<tr>
<td>Australia</td>
<td>Job control</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>Cigarettes smoked per</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td>week</td>
<td></td>
</tr>
</tbody>
</table>

Studies examining the effects of smoking on neopterin levels have shown contradictory results (see Section 3.11.6) and there was no significant bivariate correlation between
neopterin and smoking status in this sample. Some studies have however reported depression of neopterin levels in smokers – this would fit with a negative mediating effect of smoking on the inverse association between SES and neopterin levels.

For women, only the number of hours worked per week provided significant mediation, particularly for the association between serum neopterin levels and income and occupational measures of SES.

Table 5.28. Summary of significant mediation between SES and serum neopterin

<table>
<thead>
<tr>
<th>Mediating variables</th>
<th>Personal income</th>
<th>Personal occupational level</th>
<th>Personal occupational status</th>
<th>Personal educational level</th>
<th>Household income</th>
<th>Household equivalent income</th>
<th>Household occupational status</th>
<th>Communtiy position</th>
<th>Position in Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Males</strong></td>
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<td></td>
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<tr>
<td>Cigarettes per week</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td></td>
<td>X</td>
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<tr>
<td>Economic strain</td>
<td>X</td>
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<tr>
<td>Social capital</td>
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<td></td>
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<tr>
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<td>Hours worked per week</td>
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<td><strong>Females</strong></td>
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<td>Hours worked per week</td>
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<td>X</td>
<td>X</td>
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<tr>
<td>Job control</td>
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<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low control, low demands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Multiple regression analysis

Males: there was no statistically significant association of any SES variable or combination of SES variables with neopterin levels in this sample. A model constructed using the behavioural, psychosocial and biological variables contained only decision authority, weight, job security, time taken to relax in the last month and sense of job fairness (p = 0.005, Adj $R^2 = 0.09$). Of these variables, the strongest association was for any level of job security (compared to “not at all secure”, beta = 0.38), and any level of relaxation time (compared to none, beta = 0.22).

Females: The simple model, including only SES, terms included only personal occupational status and education (p = 0.0008, Adj $R^2 = 0.10$). Higher levels of neopterin were associated with lower personal occupational status and lower SES as measured by educational level. The full model consisted of job strain (low control/high demands), BMI and vigorous physical activity (p<0.0001, Adj $R^2 = 0.23$). Lower frequency of vigorous activity (beta = 0.32) and higher BMI (beta = -0.28) were associated with higher levels of neopterin (note that the signs refer to the association with the transformed variable and thus are in the opposite direction to the association with the non-transformed variable). Having low job control and high demands was associated with a higher level of neopterin than any other combination of job demands and control (beta = -0.22). Occupational status had a strong (negative) independent association with neopterin (beta = 0.32), as did having a postgraduate degree (lower neopterin), compared to having only a secondary education (beta = 0.44).
5.6.5 Salivary IgA

The normal range for salivary IgA concentration in adults (fasting, non-stimulated) is 20-500 mg/L. In this study the median (range) was 301.9 (33.4-2000) mg/L. 30.4% of samples fell outside the normal range. (Although this represents a high proportion of results outside the normal range, results were double-checked and are correct).

The distribution of values of salivary IgA concentration was positively skewed (skewness = 1.76), but was normalized with a log transformation (skewness = -0.017).

Figure 5.9 Transformation of salivary IgA

Variation with SES

The correlations of salivary IgA with measures of current SES were weak and inconsistent in direction, for both genders.
Table 5.29 Correlation between salivary IgA and SES

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Personal income</td>
<td>-0.05</td>
<td>0.49</td>
</tr>
<tr>
<td>Household income</td>
<td>-0.03</td>
<td>0.72</td>
</tr>
<tr>
<td>Equivalent household income</td>
<td>-0.02</td>
<td>0.80</td>
</tr>
<tr>
<td>Occupational level</td>
<td>0.01</td>
<td>0.85</td>
</tr>
<tr>
<td>Personal occupational status</td>
<td>0.03</td>
<td>0.71</td>
</tr>
<tr>
<td>Household occupational status</td>
<td>0.01</td>
<td>0.88</td>
</tr>
<tr>
<td>Educational attainment</td>
<td>-0.03</td>
<td>0.64</td>
</tr>
<tr>
<td>Accommodation type</td>
<td>0.0009</td>
<td>0.99</td>
</tr>
<tr>
<td>Number of cars in household</td>
<td>-0.14</td>
<td>0.06</td>
</tr>
<tr>
<td>Perceived position in community</td>
<td>0.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Perceived position in Australia</td>
<td>0.02</td>
<td>0.75</td>
</tr>
<tr>
<td>Mother’s educational level</td>
<td>-0.10</td>
<td>0.17</td>
</tr>
<tr>
<td>Father’s educational level</td>
<td>-0.15</td>
<td>0.04</td>
</tr>
<tr>
<td>Father’s occupational level</td>
<td>-0.08</td>
<td>0.28</td>
</tr>
<tr>
<td>Father’s occupational status</td>
<td>-0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Childhood household occupational status</td>
<td>-0.13</td>
<td>0.09</td>
</tr>
<tr>
<td>Composite SES</td>
<td>0.004</td>
<td>0.97</td>
</tr>
</tbody>
</table>

For males, the strongest associations were with measures of childhood SES with higher SES associated with lower levels of salivary IgA. A similar pattern was not seen in women. The only statistically significant association for women was with educational attainment (increasing education associated with decreasing sIgA concentration) – this is also a measure of child and early adult SES, rather than current SES.

**Variation with other measures**

**Behavioural variables:** Salivary IgA concentration showed no statistically significant association with measures of alcohol intake, physical activity or smoking status. There was however, a statistically significant negative association with number of cigarettes smoked per week, for women only (r=-0.22, p = 0.006).
Strain measures: There were no statistically significant associations between salivary IgA concentration and any measure of economic strain, perceived stress, or total recent life events.

Psychosocial variables: There were no statistically significant associations between salivary IgA concentration and any measures of personality, coping style, social capital or social support.

Biological and health variables: No biological variables showed a significant association with salivary IgA levels in men in this sample. In women, salivary IgA concentration was positively and statistically significantly associated with diastolic blood pressure ($r = 0.16, p = 0.04$) and had a negative, statistically significant association with serum cholesterol ($r = -0.17, p = 0.04$), HDL cholesterol ($r = -0.18, p = 0.03$) and LDL cholesterol ($r = -0.18, p = 0.03$).

Work-related variables: There was a positive, statistically significant association between salivary IgA concentration and job strain (low control, high demands compared to high control, high demand work) in men ($p = 0.03$) but not in women. Work variables had otherwise no statistically significant association with IgA concentration, as measured in the current study.
Mediation analysis

Tables 5.30 and 5.31 present the results of mediation analysis of the association between salivary IgA concentration and measures of socioeconomic status.

In males, elements of the work environment provide significant mediation of the SES/biomarker association, despite the lack of a significant association between IgA concentration and SES in bivariate analysis.

In females, only cigarette smoking provides any important mediating effect, but this generally fails to reach statistical significance.

Table 5.30 Mediators of the association between salivary IgA and SES.

<table>
<thead>
<tr>
<th></th>
<th>Males Mediating variable</th>
<th>p</th>
<th>Females Mediating variable</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Personal income</td>
<td>High job control, high demands</td>
<td>0.027</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Household income</td>
<td>High job control, high demands</td>
<td>0.028</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Equivalent household income</td>
<td>High job control, high demands</td>
<td>0.033</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>Fasting glucose</td>
<td>0.058</td>
<td>Number of cigarettes per week</td>
<td>0.077</td>
</tr>
<tr>
<td>Occupational level</td>
<td>High job control, high demands</td>
<td>0.040</td>
<td>Number of cigarettes per week</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>Job control</td>
<td>0.066</td>
<td>Number of cigarettes per week</td>
<td>0.042</td>
</tr>
<tr>
<td>Personal occupational status</td>
<td>High job control, high demands</td>
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<td>None</td>
</tr>
<tr>
<td></td>
<td>Job control</td>
<td>0.036</td>
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</tr>
<tr>
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<td>High job control, high demands</td>
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</tr>
<tr>
<td></td>
<td>Job control</td>
<td>0.049</td>
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<td>None</td>
</tr>
<tr>
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<td>None</td>
</tr>
<tr>
<td>Accommodation type</td>
<td>None</td>
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<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Number of cars in household</td>
<td>None</td>
<td>0.051</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Perceived position in community</td>
<td>Vigorous physical activity</td>
<td>0.051</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Perceived position in Australia</td>
<td>None</td>
<td>0.051</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Table 5.31. Summary of significant mediation between SES and salivary IgA

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mediating variables</td>
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</tr>
<tr>
<td></td>
<td>Personal income</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Personal occupational level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Personal occupational status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Personal educational level</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Household income</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Household equivalent income</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Household occupational status</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Community position</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Position in Australia</td>
<td></td>
</tr>
<tr>
<td>Vigorous activity</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>High control, high demands</td>
<td>X  X  X  X  X</td>
<td></td>
</tr>
<tr>
<td>Job control</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarettes per week</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multiple regression analysis

Males: There was no statistically significant association with any SES variable or any combination of variables, for salivary IgA concentration.

Females: The simple model for SES terms in women included income, household income, occupational level, highest occupational status in the household and number of cars in the household (p = 0.004, Adj R² = 0.10). Notably, income, occupational level and household status have negative coefficients (increasing SES associated with decreasing IgA concentration) while household income has a positive coefficient (increasing SES
associated with increasing IgA concentration). Having one car compared to having no car
is associated with a higher concentration of salivary IgA, but having two or more cars is
associated with a lower IgA concentration. Income and household income are likely to be
strongly collinear and perhaps the opposite signs reflect one making up for the inclusion
of the other, but the model is significantly worse if neither is included, or if either one is
excluded. Perhaps for women, having a low personal income is associated with a high
concentration of salivary IgA, but the combination of low personal income in a home
with high household income is particularly adverse to IgA levels.

The full model included the SES measures already described as well as sense of job
fairness (how fair the pay benefits and conditions are for the work done), number of
cigarettes per week and systolic blood pressure. The final model was statistically
significant (p<0.0001, Adj R² = 0.23). Household occupational status had the strongest
association with salivary IgA (beta = -0.42), while household income (beta = 0.26),
income (beta = -0.19), occupational level (beta = 0.29), number of cigarettes per week
(beta = -0.16) and systolic blood pressure (beta = 0.16) also showed statistically
significant independent associations.

5.7 Conclusion

This chapter has presented the results of the Biomarkers Study and the analysis required
to test the study hypotheses. It has explored some characteristics of the markers themselvess to determine their suitability for use under Kelly et al’s criteria: their validity
as stable markers of the physiological status of various body systems; their associations
with behavioural, psychosocial and other biological measures and their variation with SES.

In this study sample there was indeed socioeconomic variation in some, but not all, psychosocial measures, in particular increasing SES was associated with decreasing sense of economic strain, increasing job control and increasing work demands. Weaker, but statistically significant associations were found between increasing SES and more active coping style, stronger sense of optimism, fewer major life events, more job security, stronger sense of belonging and civic trust, higher levels of social capital and social support, longer job hours and decreasing difficulty of getting another job if the current job was lost. Notably, there was no significant socioeconomic variation in a measure of perceived stress, sense of control, hostility, mental health or neuroticism.

Examination of the variation in the biological measures indicated that all measures except salivary IgA concentration showed trends in the expected direction, i.e. plasma fibrinogen, glycated haemoglobin, serum neopterin levels and waist-hip ratio decreased with increasing SES. Salivary IgA showed a weak and inconsistent direction of variation with SES. For each of those four markers that did show a consistent trend with socioeconomic status, this trend was in a direction of increasing favourability with increasing SES. Notably the strength of the association with each SES measure varied by gender: plasma fibrinogen and glycated haemoglobin were most strongly associated with SES in females and for measures of occupation and income; waist-hip ratio was most strongly associated with SES in males and for measures of occupation, education and
childhood SES; and serum neopterin was most strongly associated with SES in women and for occupational measures.

In bivariate analysis there were few associations between the biological markers and the psychosocial factors. Plasma fibrinogen was negatively associated with negative coping style in women only and with skill discretion in men. Glycated haemoglobin was negatively associated with active coping style and social support in men and positively with depression and anxiety in women. There were no associations between WHR and any psychosocial factor. Serum neopterin was negatively associated with trust and belonging in men and positively with job strain in women. Salivary IgA was positively associated with job strain in men only. In this healthy, middle-aged population there seems to be little evidence of the measured psychosocial factors affecting body systems represented by the chosen markers.

Mediation analysis suggested that the mediators of the association between the markers and SES, were relatively consistent for different measures of SES for any particular marker, but were markedly different from marker to marker. Thus the most important mediators of the association between plasma fibrinogen and SES were measures of job control, sense of economic strain, smoking status, active coping style and positive interaction with friends and family, in males. In women, frequency of alcohol intake, job control, economic strain, number of hours worked per week and sense of job fairness were the most important mediating variables. For glycated haemoglobin, only active coping style in men was important in mediating the association between glycated
haemoglobin and SES, and there were no statistically significant mediating variables in women. Although there were strong associations between waist-hip ratio and SES, none of the measured variables provided statistically significant mediation of this association. In the association between serum neopterin and SES, significant mediating variables were economic strain and number of cigarettes smoked per week in males and number of hours worked per week in females. For salivary IgA, only work demands provided significant mediation of the association with measures of SES in males, while number of cigarettes smoked per week was the most important mediating variable in females.

In general, SES explained only a small proportion of the variation in the biological markers. While the inclusion of behavioural, psychosocial and other biological measures in multiple regression models accounted for a large proportion of the variation in some of the markers, few of these other variables were significant mediators of the SES variation in marker levels. To some extent this was because those variables retained in the multiple regression models did not themselves show any SES variation. However, even in those models, e.g. WHR in males, that did contain variables that were themselves associated with SES in correlation analysis, there was no significant mediation of the SES association with the marker.

Work variables and active coping style seemed to be of particular general importance in mediating the association between these biological markers and a variety of measures of SES.
Chapter 6.
Discussion

6.1 Introduction

The Biomarkers study tested three hypotheses relating to the theory that psychosocial factors contribute to the socioeconomic gradient in health. Rather than using a measure of ill health as an outcome, I measured five biological parameters that have a plausible association with the likelihood of future disease. This allowed the study to be conducted in healthy individuals, thus avoiding any possibility of reverse causality in which reporting of psychosocial or SES factors were altered by disease status.

In addition, the five biological markers represented different body systems that may be affected by activation of the physiological stress response. If psychological stressor perception activates the stress response, then it is likely that these markers would also be altered as part of that response. Because this is a cross-sectional study, I cannot infer causation between the outcome and explanatory variables, but mediation analysis provides an indication of possible causal pathways. Longitudinal studies would be required to confirm these.

To my knowledge, no researcher has previously used physiological variables in healthy individuals as markers, in this manner. In Chapter 3, Part 2 I outlined the literature that examines each of these biological parameters against Kelly et al’s criteria for population markers of the SES-stress-disease connection [154]. I begin this chapter by discussing the
results from the Biomarkers Study that test these criteria. I then examine the results in regard to each of the three study hypotheses, with reference to pertinent literature, and conclude with a more general discussion of how this research complements other research in the health inequalities field.

6.2 Testing the markers

Kelly et al suggest that criteria for ideal markers to investigate the SES-stress-health pathway include:

1. It must be affected/caused/created by stress;
2. It should have a long half-life (months to years). It should not require repeat measurements;
3. It should be independent of demographic factors (e.g. sex or age) or else have a well-defined demographic distribution;
4. It must be relatively easy and cheap to measure (preferably with an easily reproducible, automated method);
5. It should require no special sample preparation; and
6. It should have readily available reference standards.

6.2.1 Plasma fibrinogen

The review of fibrinogen (section 3.8) confirmed that plasma fibrinogen, as part of the acute phase response, is likely to be elevated in response to activation of the neuroendocrine and immune response to stressor exposure and perception, via the sympathetic nervous system, glucocorticoids and IL-6. Fibrinogen has a well-defined demographic distribution, requires no special sample preparation and measurement,
though not routine, is common, relatively cheap and automated, with readily available reference standards.

However, fibrinogen has a relatively short half-life and substantial evidence points to wide intra-individual and seasonal variation in serum levels, suggesting that there may be problems in regard to the second criterion, for this biomarker. The Biomarkers Study also found substantial intra-individual variation and a seasonal pattern, with higher fibrinogen levels in winter months. Furthermore, this study suggests probable systematic differences in fibrinogen levels, dependent on which laboratory performed the sample analysis.

Plasma fibrinogen has a plausible association with activation of the body’s stress response, and is commonly and easily measured. However, wide intra-individual, inter-laboratory and seasonal variation, meaning that a single measurement may not accurately reflect the steady state, limits its use as a marker of chronic activation of the stress response.

6.2.2 Glycated haemoglobin

Glycated haemoglobin is plausibly increased under conditions of repeated activation of the stress response, has a long half-life and the Biomarkers Study confirms that there is little intra-individual variation. There is, however, a seasonal pattern with a peak in late autumn and a trough in late spring. Glycated haemoglobin has a well-defined demographic distribution and is relatively cheap and easy to measure. However,
measurements vary depending on the laboratory in which the samples are analysed, thus some caution is required in its use in population studies.

The stability over time, ease of collection and measurement of samples, and its plausible association with repeated or chronic activation of the stress response makes glycated haemoglobin a good potential marker for this type of investigation.

6.2.3 Waist-hip ratio

Although the evidence and hypotheses reviewed in section 3.10 indicated that increased waist-hip ratio may be caused by chronic stress, the Biomarkers study found few associations between WHR and measures of psychosocial stress. WHR has a well-defined demographic distribution, is stable over time and easily measured on a population basis, although care must be taken to ensure standardised measurements. Different populations may have a different distribution of WHR due to genetic factors [301, 492], so that its chief use is probably in comparative studies of different population subgroups, rather than as a measure of abnormality per se.

6.2.4 Serum neopterin

Physiological pathways exist whereby neopterin levels could be depressed as a result of the acute stress response, but the effect of chronic stress is less clear. The one study that has examined intraindividual variability of neopterin levels indicated that population levels were quite stable over time, but there was marked intraindividual variability. In the Biomarkers Study, the intraclass correlation for neopterin in two samples taken three months apart was only modest. Single measurements may not reflect long-term levels of
neopterin, on an individual basis, and it would be preferable to have samples taken on more than one occasion. There is a well-defined demographic distribution and sample collection, preparation and analysis is relatively easy, inexpensive and can be automated.

It is likely to be difficult to find accurate markers of the effects of chronic stress on the immune system, which by its nature is designed to respond to acute threats, whether physical or psychological. Neopterin is a general marker of activation of the cellular immune system that, unlike many of the cytokines, is produced in measurable amounts in serum. It is moderately stable over time in individuals and in population groups, and may represent the most appropriate marker of the cellular immune system currently available for population studies.

6.2.5 Salivary IgA

Theoretically, humoral immunity may be depressed by repeated activation of the stress response and there are known effects on the secretion of both saliva and salivary IgA, by stress hormones. Salivary IgA has a well-defined demographic distribution and samples are easily collected, stored and analyzed. However, there are several different collection techniques, resulting in lack of standardization of the results from different research groups. Salivary IgA concentration is affected by recent food intake, impending upper respiratory tract infections, acute stress, time since rising, smoking habits and perhaps physical activity. Results from the Biomarkers Study support previous findings of only modest intraindividual stability over time in the measures, so a single measurement may not be ideal as a reflection of the steady state in an individual.
In the pilot study, some wildly high IgA concentrations were recorded (compared to the available reference range), which were likely to be associated with infection, despite participants being instructed to defer the sample collection if they felt unwell. Such samples may have been collected in the preclinical period of an upper respiratory tract infection, and be unrelated to activation of the acute or chronic stress response.

Difficulties in ensuring that collection takes place during an infection-free time, with standardization to time since waking, food intake, physical activity and level of hydration are serious problems in the use of salivary IgA in population studies to examine associations with chronic stress and as a marker of chronic stress.

6.3 Stress, SES and Disease

The theory that psychosocial factors cause at least part of the SES gradient in health implies that psychosocial stress (e.g. job strain), or psychosocial assets (e.g. social support), are differentially distributed, by SES. Psychosocial stress could be causative of ill health either directly by affecting body systems via the stress response and promoting a general susceptibility to disease, or indirectly via health damaging behaviours, adverse material circumstances or poorer access to preventive services. Psychosocial assets would moderate stressor exposure, or the perception of stress, to decrease the deleterious effects of the stress response, on body systems. Either or both of these mechanisms could contribute to the SES-health gradient.
This study explicitly sought to measure the presence of a gradient in psychosocial factors and psychosocial assets. As the results indicate, there was indeed a socioeconomic gradient in some measures of stressor exposure, e.g. economic strain, and number of life events, with lower SES associated with increased levels of stressor exposure. In addition there was a gradient in psychosocial assets, e.g. optimism, sense of belonging and positive support from family and friends, with a gradient of increasing psychosocial assets with increasing SES. Thus psychosocial forces are acting at two points to maximize the experience of stress in lower SES groups.

In this section I discuss the results of the Biomarkers Study in the context of relevant research linking stressor exposure and experience, to SES and to disease to examine steps of the hypothesized pathway:

\[\text{SES} \rightarrow \text{Stress} \rightarrow \text{Disease}\]

Few other investigators have specifically examined the associations between psychosocial factors and SES, apart from perhaps SES variation in work characteristics. In general there is a variation of psychosocial job attributes with socio-economic status, with more adverse conditions (more demands, less control, more insecurity, less opportunity for monetary and esteem rewards) in lower socio-economic groups, whether self-reported or based on imputed job characteristics [493-495]. In the Whitehall II study, Marmot found that fewer of those men and women in lower status jobs reported control over their working lives, or satisfaction with their work situation [74]. As in the Biomarkers study, more of those in higher status jobs reported having to work at a fast pace.
However, Davey-Smith et al have raised the question of collinearity in the measurement of job control and SES, suggesting that job control may be a proxy for SES [496]. The Biomarkers Study does not support this possibility, with subgroup analysis revealing that even in the lowest quartile of occupational status, the full range of job control scores is represented. In addition, within this lower SES group there is an almost significant (p=0.06) difference in plasma fibrinogen levels between those in different job strain categories. High levels of job control are correlated with higher levels of SES, but the association with markers of ill health is present at each level of the socioeconomic hierarchy.

Not all studies have found links between SES and job stress. Using a composite job strain index that included measures of work social support, work overload, identity destructive character of work and security decreasing character of work, Matthews et al found no correlation between occupational prestige and work strain in healthy middle-aged men and women [123]. However, the elements of the work strain index were not examined separately and on the basis of other studies, including the Biomarkers Study, work overload might be expected to vary in the opposite direction to the other measures of job strain, thus creating a null correlation of the overall index, with SES.

Among women, Wamala noted that lower occupational class was consistently associated with lower job control, but an association of increasing work demands with increasing SES was apparent only for women with coronary heart disease [497]. A number of
studies have found a positive association of work demands with SES [498, 499], while several studies have found that there is an interaction between SES and workplace characteristics, so that the combination of low SES and adverse work conditions is particularly potent in explaining the increased adverse health events seen in lower SES groups [495, 500].

Job strain is associated with adverse health outcomes, but there is little consistency in which elements of job strain are associated with ill health. Ischaemic heart disease and myocardial infarction have been associated with job demands only [131], job control only [501] and with the composite measure, job strain [502]. A few studies have shown no relationship between measures of ill health and job control/demand/strain [503] while others show an inverse association between incident coronary heart disease and job strain [504]. Job strain has been associated with increased diastolic blood pressure [505], decreased testosterone [506], elevated ambulatory blood pressure [500], elevated glycated haemoglobin [507] and elevated early morning cortisol [508]. Low job control has been associated with high plasma fibrinogen [509], especially in women. Adverse work conditions have been associated with immunosuppression and increased incidence of upper respiratory infections [510].

Longitudinal studies have suggested a causal relationship between various aspects of adverse psychosocial work conditions and ill health (measured in a number of different ways) [511-513]. Some studies have indicated that the effects of job strain are particularly potent in particular segments of the population – men less than 50 (compared
to older men) [131]; lower income groups [495]; and manual workers [514]. Morrison et al point out that control is likely to be most important if it is over those particular demands that are seen as demanding: “When people perceive their jobs to be demanding they will tend to be less satisfied unless they also see their jobs to be giving them the control and support necessary to successfully deal with the demands” [515].

Not surprisingly higher SES men and women in the Biomarkers Study perceived their jobs as more secure and themselves as more marketable should they lose their job, than those in lower SES groups. Traditionally, the unskilled and least educated are most vulnerable to layoffs, but with restructuring of employment to create smaller permanent workforces there has been an increase in casual employment and contract work. Such work by its nature has uncertain prospects of continuity [516], perhaps increasing the experience of job insecurity across all occupational groups. In a Canadian survey, one third of employed persons believed that they could lose their job in the next two years, and over one third were not confident that they would be able to find an equivalent job within six months if job loss did occur (cited in [517]). In Kornhauser’s (1965) study, concern about job security is “expressed by equal proportion of men at all skill levels” (cited in [518]). There appear to be few empirical data specifically examining the association between job insecurity and socioeconomic position. McDonough demonstrated a negative correlation with income (-0.19, p<0.01) and a positive correlation with education (in years) (0.03, p<0.01) using a dichotomized measure of job security where 1 = high insecurity and 0 = low insecurity [517].
Perceived job insecurity and lack of marketability are associated with psychological distress [519], depression [518] and effects on physical health with significant increases in blood pressure among workers in lower occupational classes faced with possible job loss [520] and poorer self-rated health [517] in cross-sectional studies. In longitudinal studies, job insecurity was associated with increased hospital admissions [521], and the development of increased risk factors for cardiovascular disease [516, 522, 523]. Heany showed that chronic job insecurity was associated with increased physical symptoms compared to job insecurity measured at a single point in time [524] but this was not confirmed in the Whitehall II studies [523]. Personality modifies the impact of job insecurity, with positive personality disposition (congruent to optimism) lessening, and negative personality disposition (negative affect) worsening, the impact of job insecurity on psychological distress [525].

It seems hardly surprising that economic strain is associated with lower SES, and might even be considered a proxy for SES. Economic strain is a subjective measure of the impact of low SES on the individual. Indeed the variable has a higher correlation with subjective social status (perceived position in Australia) than with any other measure of childhood or adult SES in this study, except household income. However, a correlation coefficient of −0.39 (between composite SES and the economic strain variable available for the combined studies) suggests that SES and economic strain are not automatic partners.
Ullah postulates that perceived financial hardship may be more important than objective measures of income in determining health outcomes [526]. Power found that the adjusted odds ratio for experiencing financial hardship in social classes IV and V (lowest) compared to social classes I and II was 2.02 (p<0.05). Financial hardship was, in turn, associated with increased psychological distress, particularly in women [527]. Economic strain is a critical mediator of the path from job loss to poor physical and psychological health [528, 529] and the combined experience of being in a lower SES group and having a severe impairment due to illness increased the odds ratio for experiencing financial hardship (compared to a higher SES group) from 2.3 to 8.0 [530]. In a cross-sectional study of unemployed men and women, financial hardship was positively associated with both emotion-focused coping and emotional distress, but all were less frequent among those who had been unemployed for more than three years [531]. Longitudinal studies are required to clarify the importance of coping style in the route between objective and subjective financial hardship.

In the Biomarkers Study active coping style was positively correlated with socioeconomic status. There is evidence of modest genetic influences on coping style and gene-environment interactions are likely [532]. Adverse coping styles, including self-distraction, thought suppression and suppression of feelings, were negatively correlated with income and education in survivors of Hurricane Andrew [533] and problem focused coping was less frequent among subjects with low education and perceived financial hardship [531]. While several studies have examined the impact of different coping styles on psychological and physical ill-health [534-536], the association with SES in these
studies is not documented. In general passive, avoidant coping styles are associated with poorer health outcomes [534], while active, problem focused coping is associated with fewer hospital admissions [535], longer survival after melanoma diagnosis [536] and improved immune status [537].

Sense of optimism increased with increasing SES in this study sample. Several studies have found a stronger association between SES and the negatively worded items on the LOT compared to the positively worded items, i.e. the variation in SES is a variation in the expectation that fewer negative events will occur, rather than the expectation that more positive events will occur [452, 538, 539]. This finding is not confirmed in the Biomarkers study, with positive and negatively worded items having a similar correlation with SES.

Increased optimism has been associated with decreased susceptibility to colon cancer [538, 540], faster recovery following a heart transplant [541], and more favourable adjustment to important life transitions [542]. In the Kaunas-Rotterdam study, Lithuanian men were much less optimistic than men in Rotterdam, and this factor statistically explained 30% of the excess in all-cause mortality seen in men in Kaunas (cited in [119]). Pessimism is associated with several negative health outcomes (for review see [452]), but there is some inconsistency in the association of optimism and pessimism with positive and negative health outcomes, including the effect of optimism/pessimism on immunocompetence in response to stress [452]. Furthermore at least part of the effect of optimism on health is through a close connection with an active coping style [451].
Social capital was assessed in the Biomarkers study using two questions, one relating to trust, and the other to how helpful other people were. SES was most strongly correlated with trust. Lochner stresses that social capital is exclusively a community level measure; aggregated individual responses are used as an explanatory factor for the ecological association of income inequality to poor health [543]. However, Berry et al develop the notion of personal social capital to examine the connection of individuals to their communities [544]. In the current study, social capital is measured as an attribute of an individual, recognizing that at this level there is a strong association with optimism and positive social support. Studies of community level social capital show clear associations between increased social capital and health of communities [447, 545], while initial studies using the concept of personal social capital show a protective effect for general psychological health [544]. Sense of belonging in the community (measuring the perceived availability of people one can do things with) was significantly stronger in higher socioeconomic status individuals.

Social support was tested with two items: positive interaction with family and friends, and negative interaction with family and friends [443]. Only the positive interaction elements showed a significant socioeconomic variation with higher support correlated with higher SES. This is a measure of the functional rather than structural components of social support and particularly examines emotional support.
Several other studies have shown a positive correlation between social support and SES [124, 546, 547], although differences by social class are generally modest. Notably, in the Whitehall studies there were SES gradients for both positive and negative social support, operating in opposite directions.

A large body of literature supports the association of lower levels of psychological distress with higher levels of emotional support [513, 546]. Much of the early work examining links between social support and physical health used measures of instrumental support and showed links between social isolation and increased mortality [37, 548, 549]. Higher levels of emotional social support are associated with improved survival after a myocardial infarct in the elderly [550], and post-stroke prognosis [551]. However, there is a paucity of research examining social support and disease incidence. In a recent cross-sectional study, Sorkin noted that lack of emotional and structural social support was associated with a greater prevalence of coronary heart disease in the elderly [552] and there is some support for deleterious effects of low social support on physiological outcomes, e.g. cardiovascular reactivity [553], CD4 counts in men with HIV [554], and HPA and SNS reactivity to stress [554].

In the Biomarkers Study, there is a negative correlation between the number of recent life events suffered by the participant or his/her partner, and socioeconomic status. Several studies have found a similar association in youth [555, 556], pregnant women [557], and community samples [558, 559].
Stressful life events have been causally implicated in the development of appendicitis [560], exacerbations of multiple sclerosis [561], onset of breast cancer [562], progression of AIDS [563] and myocardial infarction [559]. There is substantial literature on the association of recent life events with subsequent depression, with Brown and Harris calculating a population attributable risk of 73.1% for the relationship of severe life events and major difficulties, to the onset of cases of depression [560].

More recently, the effect of negative life events on physiological parameters has been studied. Stressful events in healthy subjects resulted in alterations in levels of TNF-α [564], and observation of new brain lesions on magnetic resonance imaging in patients with multiple sclerosis [565]. There have been conflicting results from studies investigating the association of life events with risk factors for cardiovascular diseases, with some studies finding positive correlation with systolic and diastolic blood pressure and serum lipid levels, but others finding negative or null results [566]. Consistent associations have been observed between life events and smoking, alcohol consumption and lack of leisure physical activity [566]. In addition, interactive deleterious effects on health of the combination of low SES and negative life events have been noted [567, 568].

The reward scale of the BAS examines the extent of positive feelings in the anticipation of a reward. In the Biomarkers Study there was a positive association with SES while there was no statistically significant association with other elements of scales measuring affect. Reward responsiveness predicts happiness and is moderately correlated with
extraversion, positive affectivity and positive temperament, but not optimism [457]. Several studies have noted a negative correlation between SES and aspects of negative affect [569, 570] but few studies seem to have explored the socioeconomic variation of positive affect. Similarly links between health and affect have largely explored associations between negative affect and health [571] rather than the possibly protective effects of positive affect.

### 6.4 Biological Markers and SES

Kelly et al state that initial conditions for markers of the SES-stress-health pathway are that they should be:

- sensitive to systematic, long term differences in socioeconomic status and living conditions
- they must be feasible to measure in large scale population surveys.

This section explicitly examines the first condition, i.e. that the markers vary by socioeconomic status.

I found significant SES variation in four of the markers in the expected direction, i.e. increasing SES is associated with more favorable levels of the biological markers. This is remarkable in a population of healthy, middle-aged men and women, living in a small city with good access to medical care, most owning their own homes and at least one car. It is a relatively affluent group, in a relatively affluent city, and yet these biological factors vary significantly with SES.
Substantial and somewhat consistent evidence exists of an inverse relationship between socioeconomic status and plasma fibrinogen, using a number of different indicators of SES, although in some studies major cardiovascular risk factors and health-related behaviours accounted for part of this social gradient in fibrinogen [182]. In the Whitehall II study of men and women aged 45-55 years, several measures of childhood SES (adult height, father’s social class and participant’s education) were inversely associated with adult plasma fibrinogen [196, 199]. In addition, adult SES (indicated by employment grade) was inversely associated with plasma fibrinogen in men (difference between high SES and low SES of 0.22g/L (95% CI 0.13-0.31)) and women (0.37g/L (CI 0.18-0.56)).

In the Kuopio Ischaemic Heart Disease Risk Factor Study, there was an age-adjusted inverse association between levels of plasma fibrinogen and four of five indices of SES: current income, education, lifetime occupational status and current material possessions. After adjustment for covariates (alcohol consumption, BMI, physical fitness, smoking, coffee consumption, HDL, LDL, blood leukocyte count and prevalent disease) the association persisted for education, current income and lifetime occupation. Analysis of the joint effect of childhood and adult SES indicated that those who were economically disadvantaged at both times had the highest fibrinogen levels but the fibrinogen levels of those who were not poor as adults showed no variation by childhood SES, in men and women [218].

In separate studies, Myllykangas and Woodward demonstrated an inverse association between SES and plasma fibrinogen, but this association disappeared after adjustment for smoking in men, but not in women [200, 226]. Wilson found that the SES variation in
fibrinogen was strongest for income, followed by education and then occupation [218], while Brunner showed that fibrinogen concentrations were lower in owner-occupiers than in those in rented accommodation [196]. The educational variation in plasma fibrinogen in women less than 65 years old was markedly weakened by adjustment for psychosocial stress and lifestyle patterns [228].

There is little direct research on the association of levels of glycated haemoglobin with socioeconomic status. Daniel et al [251] examined levels of HbA1c in indigenous populations in Australia and Canada and compared these to non-indigenous populations in Melbourne. Mean HbA1c concentration was 18.2% higher for indigenous people, but this figure was largely influenced by much higher levels of HbA1c among indigenous diabetics. While blood glucose and 2-hour post-load glucose concentrations were controlled for, it is likely that indigenous populations would experience poorer diabetic control (reflected in higher HbA1c) that may not be reflected in a single fasting glucose or glucose tolerance test at a clinic. In addition, the indigenous populations were from remote rural areas, whilst the non-indigenous populations were urban-dwellers. While some of the variation in HbA1c concentration may have been due to differences in socioeconomic status, it is difficult to apportion the variation between the various other differences between these groups.

In a survey of Jamaican school children, glycated haemoglobin was directly related to shortness at birth as well as fatness in childhood, but there was no independent association between socioeconomic status and HbA1c [572].
A number of studies undertaken to examine diabetic control have measured socioeconomic status and HbA$_{1c}$ simultaneously, using HbA$_{1c}$ as a monitor of diabetic control over time. While Frindik [252] and Larsson [250] showed lower levels of glycated haemoglobin in diabetics of higher socioeconomic status, probably reflecting higher education levels contributing to improved control, Baumer found no effect of social disadvantage on either diabetes prevalence or outcome, in childhood. Diabetes has such an overwhelming effect on HbA$_{1c}$ that results from the diabetic population are not transferable to the non-diabetic population [573].

In the Biomarkers Study, there were strong, consistent negative associations between WHR and SES, particularly in men and for occupational measures and education, as well as measures of childhood SES, e.g. parental occupational status.

Previous research has shown that in women, central fat deposition and BMI have a reliable negative association with both socioeconomic status of childhood as well as current socioeconomic status, measured in a variety of ways [286, 306-308]. Thus high WHR is linked to lower social class [265], lower educational level [265, 301], poorer income [226], partner’s occupational level [311] and personal occupation [265, 301]. Several studies indicate that the association is strongest with education level [301, 311] and that this is a graded relationship [311], although Adler suggests that subjective social status may have a stronger association with body fat distribution than objective indicators [314]. Lapidus demonstrated that low status employed women were 1.4 times as likely to
be overweight as high status employed women [312] and women working outside the home had significantly lower WHR than housewives [297].

Around 30% of studies in men report a direct (rather than an inverse) relationship [308] between measures of SES and WHR. However, most studies report an inverse relationship with SES, as measured by personal income [226] or education [256]. Marmot reported that father’s educational level, but not mother’s, was inversely associated with WHR in men; but parents’ education was not associated with WHR in women [310].

Some studies suggest that there is no association between WHR and employment status [312], but others have found that unemployed individuals had higher WHR than employed individuals [290, 304]. There are inconsistent results for an association between WHR and housing type in men and women [297].

The majority of studies have been carried out in developed Caucasian countries (USA, UK, Australia etc) and these results may not be generalisable to other cultures or races. For example, Martikainen showed that in Japan, both BMI and WHR are positively associated with SES (by employment grade) and there is no significant association with education [313].

In Australian adults (20-69 years) there is regional variation in mean WHR, with the lowest average WHR for men in Melbourne and Canberra and for women in Darwin and
North Sydney. The highest average WHR for men was in Brisbane and Darwin and for women, Melbourne and South Sydney [301].

The association between central obesity and SES is susceptible to reverse causality, where obese individuals become part of lower SES groups, because of decreased employment opportunities, ill-health etc. However, a limited number of longitudinal studies indicate that low SES predicts weight gain and risk of obesity [312]. In addition, the strong, graded relationship of WHR with education (often taken as a measure of early SES) provides some evidence against reverse causation. SES gradients in central obesity are present in children (after controlling for perinatal health), with some studies suggesting that rather than low SES children being overweight, upper SES children are lean [308]. Upward mobility does not necessarily mitigate or reverse the adverse effects of low childhood SES on adult health, i.e. adults who grew up in lower SES households had higher WHR as adults, even after adjustment for adult SES [309]. However, women with only secondary schooling who moved up the social scale had consistently lower WHR than women who married down [281].

Brunner et al demonstrated a monotonic trend in the odds ratio of having an elevated WHR by employment grade in the Whitehall studies [287] and there was a clustering of risk factors for the metabolic syndrome in the lower grades of the civil service for both men (OR=2.2) and women (OR=2.8). Despite health promotion campaigns the SES gradient in WHR does not appear to be decreasing [306], and health related behaviours
such as smoking, alcohol consumption and exercise level make only a small contribution to this inverse association [287].

To my knowledge, the Biomarkers study is the first to examine the socioeconomic distribution of serum neopterin. While there was no statistically significant association with SES in men, a socioeconomic gradient was evident in women, particularly for occupational measures.

No studies have noted the presence of variation in salivary IgA concentration with socioeconomic status. Evans et al [425] showed that lower social class (as measured by the occupational social class of the head of the household) was associated with lower salivary IgA secretory rate. However, much of this social class difference could be explained by differences in smoking rates between the classes [425].

### 6.5 Variation of markers with psychosocial factors

The five biological markers were chosen because they could theoretically be affected by the physiological stress response. However, for at least some of these markers, substantial research has been conducted on actual associations with stressor exposure and the effects of psychosocial assets. My research has also examined these links.

In the Biomarkers Study, plasma fibrinogen levels were higher in women reporting economic strain and in men reporting increased skill discretion. Other researchers have noted consistent positive associations between acute psychological stress and plasma
fibrinogen [182, 229, 230], but less consistent associations with measures of chronic stress. Raikkonen found a decrease in fibrinogen with long-term mental stress [229], while others found no change or an increase [182]. A review of 18 studies examining associations between chronic psychological stress (as measured by job strain) and haemostatic variables found that, compared with controls, chronic stressor exposure was associated with an average increase in plasma fibrinogen of 8% (range 4%-30%) [182].

Several other studies have found that measures of job stress, particularly low control, monotony and under-utilization of skills, are associated with increased fibrinogen concentrations in both sexes [78, 196, 198]. In the Whitehall II study, men in the top tertile of both self-rated and externally assessed control over work had lower fibrinogen levels than those in the bottom tertile of both measures (-0.16 g/L, 95% CI (-0.07, -0.26), p<0.001) [199]. Similar results are found in studies using the Siegrist effort-reward imbalance model of job stress [231]. The current study found no association between measures of job strain and plasma fibrinogen in women, and the association with skill discretion is in the opposite direction in men, with those with increased utilization of skills having increased fibrinogen levels.

I found no statistically significant association between fibrinogen levels and any measure of social support. Helminen showed a negative association between measures of social participation and plasma fibrinogen that persisted after adjustment for covariates including age, smoking, educational level, WHR and cardiovascular health [227]. The social participation measure used largely tapped involvement in sports clubs and sporting
activities, and was also associated with higher levels of cardio respiratory fitness. When the association between social participation and plasma fibrinogen was adjusted for cardio-respiratory fitness, it was no longer significant, suggesting that there was no independent social effect. Men with high scores on overall social support had 0.20g/L higher plasma fibrinogen level compared to men with low scores, and this difference persisted after age, cardiovascular health status, education level, WHR, HDL cholesterol and cardio-respiratory fitness were taken into account. This somewhat counterintuitive difference was (statistically) explained by a smaller number of smokers among men with low overall support, compared to those with high support.

The Biomarkers Study demonstrated a statistically significant negative association between glycated haemoglobin and elements of job control and a statistically significant positive association with economic strain and number of hours worked per week. Few other studies have specifically examined associations between glycated haemoglobin and measures of chronic stress. Daniel et al postulate that the small but significant increase seen in HbA1c in normoglycemic indigenous people in Canada and Australia, compared to normoglycemic non-indigenous people was caused by the stresses of social change, low control and poorer living conditions associated with Westernisation [251]. Cesana evaluated levels of glycated haemoglobin in relation to stressful working conditions and found that printing workers exposed to noise, shift work and long job hours had HbA1c levels that were 8.64% higher than male clerical workers [574]. After controlling for smoking and alcohol intake, Kawakami found a significant positive association between a measure of job dissatisfaction and levels of glycated haemoglobin in male employees of
an electric corporation [575] and between job strain (using the Karasek demand/control model) and glycated haemoglobin in male Japanese employees in a manufacturing firm [507]. In a therapeutic trial of a psychosocial activation program in elderly people, Arnetz noted an improvement in HbA₁c levels in the stimulated group over six months [576].

Schuck investigated the use of glycated haemoglobin as a stress marker in a small group of non-diabetic medical students undergoing academic examinations [244]. He measured a large number of psychosocial variables including extroversion, neuroticism, internality, self-efficacy, anger, anxiety, social support, social constraint, coping style, as well as deriving a daily hassles and uplifts score and a global stress rating. Although there was an increase in HbA₁c during the exam condition compared to controls, there were generally low correlations between the psychosocial variables and levels of HbA₁c. In males, active coping style was negatively associated with glycated haemoglobin in the Biomarkers Study, but there was only a low correlation between problem focused coping and HbA₁c in Schuck’s study.

Increased anxiety and depression were positively associated with increased levels of glycated haemoglobin in women in the Biomarkers Study. This finding has some support in the literature, with Theorell reporting a similar association with anxiety in a longitudinal study of relatives of cancer patients. He found that a high level of anxiety was associated with rising levels of HbA₁c during monthly assessments [247].
Positive interaction with family and friends was associated with lower glycated haemoglobin, and negative interaction with family and friends higher glycated haemoglobin, in males only, in the Biomarkers Study. Few studies have examined the influence of social support on levels of glycated haemoglobin in non-diabetic individuals. Schuck reported a low inverse correlation between HbA$_{1c}$ and levels of social support ($r = -0.10$) in his study of academic stress in medical students [244]. Improved glycaemic control in diabetics may be associated with a perception of stronger family support, however, such studies may not be generalisable to the non-diabetic population [577].

Previous research has documented associations between elevated WHR and cynical distrust in men and women [578], depression [254, 578, 579], hostility [274, 287], economic strain [580], Type A behaviour [274], anger [274], vital exhaustion [274], lower self esteem [254], and extraversion [263, 297]. Women with low BMI and high WHR had the most negative profile – greater pessimism, negative affect, and avoidant coping [254].

Women with high WHR found laboratory stressors more challenging and stressful than those with low WHR (across a range of BMIs), secreted significantly more cortisol in relation to stressor exposure and the cortisol response failed to habituate to stressor exposure [254, 315]. Men had a higher desire for control and greater cortisol reactivity when faced with uncontrollable stress than either high or low WHR women and this was most marked in men with central obesity [271].
However, in the Biomarkers Study, I found no association of WHR with any measure of psychosocial stress except for skill discretion at work, which was statistically significant in men and women, and the combination of low job control and high job demands at work, in men. In addition, there was no association with social support, belonging or social capital. Few studies have examined the association between WHR and measures of job strain. Van Amelsvoort et al observed increased WHR and BMI in association with the number of years in shift work, which persisted after adjustment for age, physical activity and smoking status [581], while Georges reported a significant association between job control and abdominal obesity, which became non-significant after adjustment for education (cited in [582]). Steptoe et al found job control did not differ in groups of teachers defined by high or low WHR status, but men with high WHR and low job control exhibited heightened stress-induced physiological activation with increased heart rate and ambulatory blood pressure [582].

Lack of social support and social isolation have been associated with increased risk for the metabolic syndrome in men and women [317], and longitudinal studies suggest that lack of perceived social support precedes an increase in central body fat [317]. In the Whitehall II studies, strain in social relationships was associated with higher WHR, as was perceived inequality, in women [310].

In the Biomarkers Study, serum neopterin had a statistically significant positive association with job strain (low control/low demands and low control/high demands) and economic strain in women. Increased levels of trust and belonging were associated with
decreased neopterin levels. These results, as well as the SES variation in neopterin levels, suggest that increased strain is associated with increased levels of neopterin, rather than the decrease predicted (and observed) in acute stress.

Elevated neopterin levels have been noted in anorexia nervosa [304], depression [357, 374-377], and seasonal affective disorder [374], with conflicting results for schizophrenia [360, 377, 378]. In contrast, depressed neopterin levels were found in post-traumatic stress disorder [368], and following acute stressor exposure [318, 367, 583]. Dunbar noted that the lowest neopterin levels coincided with the period of maximum self-perceived stress, and there was a slow return to baseline levels (>six weeks) [367].

De Gucht examined groups defined by self-perceived high and low levels of chronic occupational stress and found no significant differences in neopterin levels until the groups were separated into those with low, and those with high, psychopathology (anxiety, depression). There was a marked and significant elevation of serum neopterin in those with high stress and low psychopathology [373]. Interestingly, changes in other measures of immune function, e.g. CD8+CD11b+, were more marked in the group with high stress/high psychopathology, leading the authors to conclude that in the presence of chronic stress, different psychological outcomes are associated with specific immune function changes [373].

Only one study has investigated the effect of social support on neopterin levels, and found no association [329].
The association of stressor exposure with either concentration or secretory rate of salivary IgA has been extensively studied using acute (arithmetic tasks, cold pressor tests etc), subacute (academic examination) and chronic stressors (occupational stress, chronic caregiving). In general, salivary IgA is increased during and directly following acute stressor exposure [392-394], although there are some studies reporting decreases in salivary IgA measures [426, 427, 433]. Following sub-acute stressor exposure, most studies report decreased levels of salivary IgA, with concentration showing stronger associations than secretory rate [412, 415, 417, 430, 584]. However, increases in salivary IgA concentration with examinations [429] as well as minimal change in IgA levels [409] have been reported. Exposure to chronic stressors has been associated with depression of salivary IgA concentration, [434, 435], with some inconsistent results showing increases in salivary IgA concentration and secretory rate [432] or no difference [431].

Both concentration and secretory rate of salivary IgA are consistently increased following activities promoting relaxation and induction of a positive mood [403, 436-439]. Techniques include Reiki touch healing [437], relaxation techniques [436, 438], a nursing back rub [439] and mood manipulation using mental recall or music [403, 585].

Although I was unable to demonstrate any significant association of any personality factor with salivary IgA concentration in the Biomarkers Study, inverse associations have been noted, under conditions of high stress, with internal locus of control [431, 436], and passive coping [381]. While at first glance these findings may appear inconsistent,
persons with a high locus of control and with passive coping skills may feel similarly more threatened under conditions of high stress, with resultant depression of humoral immunity.

Pleasurable social events and increased social support (during stressor exposure) have been associated with an increase in salivary IgA concentration and secretory rate [384, 415].

### 6.6 SES, psychosocial factors and biology.

The third hypothesis to be tested in this thesis states that the measured psychosocial factors mediate the association between SES and each biological marker. To test this hypothesis, I used mediation analysis. This form of statistical analysis is more commonly used to analyze prevention programs and the factors that mediate their success or failure. As noted in Methods 4.13.1, both positive and negative mediation are possible, the latter resulting from a difference in sign between the coefficients of the regression equations involved (equivalently, if $\tau$ is increased relative to $\tau'$, rather than decreased). However, negative mediation has little intuitive meaning. Particular problems also arise if there is collinearity between variables in the full regression model, having opposite signs due to their inclusion in the same model. The proportion of the total mediation that is contributed by each variable may be opposite in sign to collinear others, e.g. in the association of serum neopterin with current educational level, mother’s education and father’s education are highly correlated and result in opposite directions of mediation (-2% for mother’s education; 31% for father’s education).
While MacKinnon recommends the use of absolute values to calculate the proportion of effect mediated [486], there is no rationale for doing this. This is unfortunate, as it would be advantageous to assess the relative contributions of various factors to the total mediation. The calculation of statistical significance involves squared terms, so that the sign of the coefficients is not a problem. The results presented and the following discussion is thus based on statistical significance of the mediating effect, rather than proportion of the total mediation.

Psychosocial factors did provide significant mediation of the association between each biomarker and measures of SES, but which particular factors were important mediators differed by gender and for different markers.

The association between plasma fibrinogen and SES was largely mediated by work variables, behavioural factors, such as smoking and alcohol intake, and the use of an active coping style. Although there was no independent association between plasma fibrinogen and perceived stress, the latter did mediate the association between fibrinogen and several measures of SES. Several authors have recommended the use of different measures of SES in research, as each measures something slightly different [90, 586]. However, the mediators of the SES-marker association are surprisingly consistent over different SES measures, for any particular marker.
Although the strength of the mediation varied by gender for different SES measures, overall the actual mediators for a particular biomarker were similar for males and females. Thus, the association with income, household income and subjective social status showed particularly strong mediation from psychosocial factors for plasma fibrinogen in males, while the same is true for equivalent income in women, with occupational level and occupational status showing similar associations in men and women. Perhaps signs of status, such as income, household income and occupational measures have more effect on male physiology. Equivalent income is a measure of household income adjusted for family size and as such is a closer measure of income actually available to the family unit. Women are more affected by their ability to purchase family goods and the affordability of resources for children, than men who may be more distant from this need.

Mediation by psychosocial factors involves a mix of psychosocial variables – it is not just stressors but psychosocial assets that are important in the SES association with fibrinogen. Thus economic strain, perceived strain, job control, and number of hours worked per week are statistically significant mediators, as are positive interaction with family and friends and use of an active coping style. In addition, sense of belonging, and trust in others in the community mediates the association between fibrinogen and some measures of SES, in men and in women.

Few factors mediated the association between SES and glycated haemoglobin. Indeed, none of the measured psychosocial or behavioural factors mediated this association in
women, despite the bivariate correlation between glycated haemoglobin and SES being stronger for women than men. As for the mediation analysis for fibrinogen, the factors providing mediation were rather consistent, over a range of measures of SES. In men, only active coping style provides statistical mediation of the association between glycated haemoglobin and SES.

Despite a strong association between WHR and SES in males, none of the measured factors provide significant mediation of the association with the traditional measures of SES. Depression is a significant mediator of the association of WHR with the subjective measures of SES, but not of more objective measures of SES. Depression may alter the reporting of perceived SES, although Singh-Manoux found that subjective social status, using the same measure as that used in the Biomarkers Study, was free from psychological biases [477]. There are clearly interconnecting pathways between subjective SES, depression and WHR, but the direction of these pathways cannot be determined in this cross-sectional study.

In women, consistent, statistically significant mediation for the association of WHR with SES is provided only by body mass index, although skill discretion is almost significant for some measures of SES, particularly occupational and income variables.

Economic strain in males and the number of hours worked per week in females are the most statistically important mediators of the association between SES and serum neopterin. This is a consistent effect across most measures of SES. Lower SES is
associated with higher levels of serum neopterin, and although this association is not statistically significant in males for any measure of SES, it is mediated mainly by economic strain and the number of cigarettes smoked per week. In the mediation analysis, these two variables have opposite signs, essentially canceling out the effect of each other, possibly causing the overall association between neopterin and SES to be non-significant. This highlights the importance of examining mediators, even when the overall association between two variables is non-significant. In traditional regression analyses, we may adjust for possible “confounders” such as smoking (e.g. of the association between fibrinogen and SES) and report that the association is no longer significant after adjustment. Such analysis misses the important mediating effect of smoking on the association between plasma fibrinogen and SES – rather than reporting that there is no independent SES association with plasma fibrinogen, it is valuable to note that smoking mediates this socioeconomic variation.

For salivary IgA, there are no significant mediating variables for the association between IgA concentration and SES in women, while in males, the most statistically important factors are work related, particularly job control and demands. Previous studies examining the association between salivary IgA and stress have generally used acute laboratory, or subacute examination, stress in a small sample of volunteers. Chronic stressors in population studies have included work stress [432], low SES [587], exposure to natural disasters [434] and over-wintering in Antarctica [435]. In Zeier’s study of air traffic controllers, increased work stress was associated with increased salivary IgA concentration, but there was no correlation with cortisol levels and the authors postulated
that the changes in IgA concentration were associated with positive emotional
generation, rather than stressor perception [432].

I noted particular problems with the measurement of salivary IgA concentration. Given a
normal range of 20-500 mg/L [405] (assuming that 2-hour post-prandial collection is
‘fasting’), 30.4% of participants in the Biomarkers Study had salivary IgA concentrations
higher than the upper limit of the reference range, with marked interindividual variability.
In a longitudinal study examining the effects of exercise and competition stress in elite
swimmers, participants were used as their own controls, because of a similar wide range
of interindividual variability [588].

Gender differences in the strength and character of the mediating variables are marked. I
propose three possible explanations for this:

1. Different stressors are perceived and thus impact, differently on the stress
response and the subsequent pathways,

2. The stress response pathways are different in males and females, so that different
biological parameters are altered by different stressors,

3. The differences are artefactual.

The first two explanations are not mutually exclusive, and some evidence exists to
support each one. A great deal of research indicates gender differences in stressor
perception and response. In addition, Taylor postulates that there is an evolutionary
difference in the female stress response, which is more species preservative than the more self-preservative response that was required for male survival [161]. Social programming over the life-course will serve to modulate (and possibly amplify) any inherited gender differences in the biological stress response. In addition to innate variations in the stress response, there is compelling evidence that the first six years of life, and particularly the first three, are of critical importance in setting basic coping skills, emotional control and habitual ways of responding to events in the environment [589].

It is possible that the biological markers shown are inadequate measures of the stress response, that the questionnaires used to measure life stressors and psychosocial assets are inadequate, and that gender differences are artefactual. However, the questions used to evaluate psychosocial factors were from standard scales, with proven reliability and validity. The data provide evidence of differential distribution of these perceived (self-reported) psychosocial factors by both SES and gender. There is good physiological and empirical support for the status of these biological factors as markers of the stress response. This does not rule out artefactual explanations, but requires that serious consideration be taken of the non-artefactual possible explanations.

If gender-specific stress responses do exist, can different psychosocial factors activate alternative parts of the stress response and their subsequent pathways? Why should fibrinogen be particularly sensitive to the effects of work? Why should glycated haemoglobin be particularly sensitive to the effects of coping style? Is salivary IgA more
sensitive to acute stress and positive mood than to chronic stress, or is it just not a good reflection of chronic stress? These are questions that this thesis can raise, but not answer.

This study had two main purposes: to evaluate these five biological parameters as markers of a chronic stress response for use in research on the socioeconomic determinants of health, and to investigate the psychosocial theory of health inequalities.

For the first purpose, fibrinogen, glycated haemoglobin, WHR and serum neopterin appear to have some utility, although the association with self-reported stress as measured in these questionnaires is poor, perhaps because the questionnaires fail to capture those psychosocial factors most likely to trigger the physiological stress response.

For the second purpose, the Biomarkers Study supports a hypothesis that psychosocial factors, both stressors and assets, mediate the association between SES and these biological markers. However, there are puzzling gender differences and a lack of consistency in which particular stressors and assets are important, suggesting that assumptions whereby a universal neuroendocrine response to stress causes alteration in these markers (and possibly later disease) is too simplistic. Any particular stressor does not produce a generalized susceptibility to disease via alterations of the endocrine, haemostatic and immune systems; rather stressors may be more specific – work related stressors may be particularly related to coronary heart disease, at least partly through elevation of plasma fibrinogen, WHR or through an abnormal immune response.
Disordered glucose metabolism may be particularly affected not just by stressor exposure, but by how people cope with such exposure.

This is the first study to use mediation analysis to examine possible routes from SES to health. Sacker et al used path analysis (which has similarities to mediation analysis) to show that different dimensions of social position correlate with distinct pathways to ill-health. In a study of women, aged 20-59 years, Sacker et al hypothesized that alternative measures of SES would work through different pathways to affect self-rated health: “lifestyle” social status (as measured by the Cambridge Scale) would affect self-rated health via behavioural factors such as diet, physical activity, smoking, and alcohol intake, and social support; occupation-based social status would work through job strain; and measures of material wealth or deprivation would alter diet, social and sport participation, as well as lifestyle behaviours. The final model supported this hypothesis, but accounted for only 25% of the variance in general self-assessed health. Furthermore, of the social status measures, material deprivation had the greatest effect on health, mediated by smoking, alcohol intake, social support, physical activity and diet [590].

Mediation analysis does not investigate more upstream determinants of health. Smoking is an important mediator of the association between SES and plasma fibrinogen. But what are the important mediators of the smoking – SES association? This may be another route by which psychosocial factors may affect the biological markers but is not explored in this study. Only the immediate effects of psychosocial factors on the SES-marker association are explored. More detailed multi-level analysis would be required to
examine other pathways between SES and the markers, that may work indirectly through behaviour.

Results from the Biomarkers Study suggest that specific types of health outcomes may be mediated by particular intermediate factors and have varying associations with different measures of SES. The results do not support the concept of a stress-induced increased generalized susceptibility to disease; rather particular types of stressors may work through alternative stress pathways and be more commonly associated with particular classes of disease. During the last century, socioeconomic gradients in ill-health have persisted, despite marked changes in the main disease outcomes involved. This has been interpreted as evidence of a generalized susceptibility to disease being associated with lower SES; but perhaps there has been a change in the nature of stressors experienced by lower SES groups. At the beginning of the twentieth century disease gradients existed for infectious diseases. Stressors experienced by lower SES groups may have affected the immune system, increasing the risk of disease. With the advent of changes in the work and living environment, stressors began to work through the haemostatic system to cause socioeconomic gradients in coronary heart disease. And we have yet to see how stressors of the future may impact on human physiology.

The Biomarkers study sample was relatively small, with an engineered distribution of SES. The sample was not representative of the Canberra population, but each SES group was randomly selected from the study population (of similar SES) and should be reasonably representative of the SES group from which it derives. All participants were
Caucasian, living in a high quality social and physical environment with probably under-representation from the lowest SES groups [91]. All participants rated their own health good or better and gave no history of ongoing disease. The study results cannot be generalized to the general Australian population of this age group. However, the SES and psychosocial variation between the participants is likely to be less than that existing in the greater Australian population of this age, so these results may underestimate both the variability in the measures and the associations between them.

6.7 Conclusion

In this study, I found demonstrable socioeconomic gradients in the levels of several biological factors that are plausible markers of the physiological stress response. In addition, the data indicated socioeconomic gradients in a number of psychosocial factors, including both stressors and psychosocial assets, such as active coping skills and social support. However there were few associations between the measured psychosocial factors and any of the biological markers. The mediation analysis provides some support for a role of both psychosocial stressors and assets in mediating the association between socioeconomic status and ill health.

The lack of association between the biological markers and the psychosocial factors is surprising in view of previous work and understanding of the neuroendocrine stress response. The reasons for this are most likely to be found in the relatively small sample size, the good health of the participants and their relatively high socioeconomic status, with limited SES variability.
Several questions are raised about the pathways from stressor exposure to physiological change, in particular to explain gender differences in perception or response as well as differential physiological effects for different psychosocial exposures.
Chapter 7.

Conclusion

Health inequalities in general, and socioeconomic health inequalities in particular, have received much research interest at individual, community, state and country levels in the last three decades. At the ecological level, it is clear that the association between socioeconomic status and health is not straightforward, with some countries and states defying the usual trend, both in the direction of better health outcomes despite generally lower international SES (GDP per capita) e.g. Costa Rica, and to poorer overall health despite higher SES, e.g. the United States. The level of income inequality within countries may be important, or may be a sign of government policies that allow both income inequality and low public investment in education, health and community-level social functioning.

It seems likely that the poorer health associated with lower SES has multiple causes, with both group and individual influences. The current study concerns individual attributes and health, and aims to further knowledge of effects operating at the individual level. Previous research has suggested that there are four major routes to unequal health outcomes, each of which act at all stages of the life-course: behavioural influences, health-related selection, issues of access (to health care, adequate diet, appropriate housing etc), and psychosocial influences. These four routes do not form separate and competing pathways (although analytically they may be separated to assess their relative significance); rather they interlink and together determine health outcomes. This thesis
has focused on an hypothesis that suggests that psychosocial factors have direct, proximal (via the neuroendocrine stress response) effects on health, as well as more distal influences on the other three routes: health behaviours, health selection and the determinants of access.

The Biomarkers Study supports the psychosocial theory. Both psychosocial stressors (more prevalent in lower SES groups) and psychosocial assets (more prevalent with higher SES) mediate the socioeconomic variation in biological markers, alterations of which may predate disease onset. Particular psychosocial factors appear to be linked to specific biological measures, suggesting that there may be some variation in the pathways by which different stressors or resilience factors have a physiological affect.

John Donne wrote “no man is an island, entire of itself: every man is a piece of the continent, a part of the main”[591]. Humans do not exist somehow independent of the social, economic and political circumstances in which they live. Yet, as McMichael points out, epidemiologists have, in general, become “prisoners of the proximate”, focused on the most proximal risk factors for disease [592]. He urges that the marriage of epidemiology with the biomedical model of health requires broader reconsideration, with a stronger focus on the ecological framework in which communities and populations exist.

Nevertheless, the current study does focus on the individual, but with full recognition of the part that the larger environment plays in determining health, both directly and
indirectly through health behaviours, acquisition of health knowledge, access and use of health services and interaction with others. Somehow, the social environment “gets into” the biology of a person, affecting the health of individuals and the collective health of populations. We need to improve our understanding of each level of influence if we are to maximize the health potential of the population.

Notably, it is SES that is associated with health; that is, it is not only poverty that is linked to poor health, but there is variation in health status across the full range of SES. Certainly, those lowest on the SES ladder have the worst health as a group. But those in the middle class, though higher on the SES/health curve, are the most numerous, so the majority of those suffering sub-optimal health are not the poverty-stricken, but are from the middle class. Rose suggests that this is “one of the most fundamental axioms in preventive medicine: a large number of people exposed to a small risk may generate many more cases than a small number exposed to a high risk” [593].

Socioeconomic health inequality is a multi-level problem, requiring multi-level research (both at multiple different levels and integrated over multiple levels), and action at multiple levels. Dahlgren’s model of the causes of health inequalities (Figure 1.1) is superficially like the layers of an onion. In reality, the layers are not distinct, but are blurred by the interaction between both adjacent and distant, layers. As scientists most of us have become locked in our spheres of interest, each discipline studying one layer of the onion. Yet such study is of limited relevance – we could become experts in our particular layer and remain ignorant of the important influences of the other layers and
the interactions between them, by failing to recognize the value of multidisciplinary teams of study.

Although the Biomarkers Study was individual-based, it drew from the disciplines of psychology, sociology, endocrinology, physiology and immunology. Aspects of several different layers of Dahlgren’s schema were included: different elements of socioeconomic status, work factors, social conditions, lifestyle factors, gender and some constitutional factors. Psychosocial factors are not specifically mentioned in this schema, but probably act at several different levels. However, the final pathways of effect from all factors are at the level of individual body physiology. The Biomarkers Study used a cross-disciplinary approach to examine the factors that lie at levels between socioeconomic status and this final pathway of effect.

The future research agenda is large, but much work has already been done, albeit not explicitly within the health inequalities framework. For example, if we are to understand socioeconomic influences on health behaviours, then we need to include socioeconomic terms in the investigation of health behaviours: why do people smoke and what enables some of them to stop smoking? What are the determinants of physical activity? Of education? Of access to preventive care? If one were to draw in all the intertwining threads between the layers of the onion, we would see that many of these pathways have already been studied, but in fields distant from health. Figure 7.1 suggests some of the interacting pathways to health.
Woodward and Kawachi have asked, “Why reduce health inequalities?” [594]. They suggest four answers to this simple question:

1. Inequalities are unfair. Perhaps one of the most important evolutionary developments of *Homo sapiens* is that of a large and complex brain, giving the human advantage of abstract thinking, forward planning, language and a social conscience [4]. While, given apparent equal opportunity, individuals may be thought responsible for their own actions in regard to health, in reality the social, political and economic environment throughout the life-course shapes health-affecting decisions. Such real inequality of opportunity offends our sense of justice (but perhaps appeals to the sense of selfishness of some higher on the SES ladder).

2. Inequalities affect everyone. The work of Wilkinson, Kawachi and others suggests that marked income inequality is associated with loss of social cohesion and increased violence and crime as well as poorer overall population health (see Chapter 2). Even though the lowest SES groups suffer the worst health, the whole population fails to achieve optimal health outcomes. Woodward et al note the spill-over effects of diseases that arise under conditions of poverty and go on to affect whole populations, e.g. AIDS, cholera and tuberculosis [594].
Figure 7.1 Interacting pathways to health across multiple levels of influence

Political influences – safety nets; public education; universal medical care; old age pensions; parent support; workplace reform

Social capital, social cohesiveness, sense of community – violence, crime, social isolation

Social support, family, relationship stability

Personality – self-esteem, optimism, affect, coping skills; Stressor exposure

Health behaviours – access to health care, smoking, alcohol, physical activity, diet

Education

Occupation

Income
3. Inequalities are largely avoidable. By the shape of the income/health curve, it should be possible to reduce health inequalities without diminishing the health of the population overall. The promotion of economic growth does not mean an automatic increase in economic or health inequality, provided there is social and political will to retain public funding for education and health as well as safety-nets for the disadvantaged or those in need in times of crisis, e.g. unemployment.

4. Cost effective interventions exist. Substantial gains in population health are likely to follow from interventions that reduce health inequalities, improving the health of whole populations towards that currently enjoyed only by those of higher SES. The Independent Inquiry into Inequalities in Health (UK) showed that for men aged 20-64 years, if all social groups had the same mortality rates as the top two social classes, there would have been approximately 17,000 fewer deaths each year from 1991 to 1993 [63]. However, there is little empirical evidence of either effectiveness or cost-effectiveness from trialled interventions. Further research will be necessary to decide whether interventions to improve population health by decreasing socioeconomic health inequalities can be cost-effective (although a saving of 17,000 lives over three years in a prime working age group should justify considerable cost!).

There are two requirements for interventions to improve health inequalities: the first is a government interested in long-term gain for substantial current investment, and the second is a greater understanding of what type of intervention might work. Rose proposed “the prevention paradox” in which “a preventive measure that brings large benefits to the community offers little to each participating individual” [593]. It may be
difficult to justify (to the public) programs that appear to provide little gain to individuals, despite considerable community and population-level health gain.

Universal rather than targeted programs may be more effective (though perhaps less appreciated as such by individuals) in reducing socioeconomic health inequalities, as well as avoiding the “blame the victim” problem. Targeted interventions risk stigmatisation of lower SES people and fail to recognize that while there is a higher level of potential health loss among the poorest groups in society, in absolute terms the greatest health loss is in the middle classes (who have higher numbers but lower per person health loss). Interventions must recognize the societal influences on the “victim” and even the community level “victim”. It may not be possible to alter individual behaviour or community level attributes such as social capital without an understanding of the upstream factors that have determined the individual behaviours or the loss of social capital within that community.

Prevention, as well as intervention, at many different levels will be the key to alleviating socioeconomic health disparities. The recent Early Years Report from Ontario, Canada, recommends universal interventions across the life-course: (universal) programs to improve parenting skills to optimize neural sculpting of infants and young children, to improve maternal and infant nutrition, and to ensure that the education system teaches life skills as well as academics [589]. But, simultaneous with these interventions, the wider community must recognize the importance of improving parenting skills, support changes in the workplace to allow such skills development, and vote for governments that
provide education initiatives and adequate welfare support, to allow universal access to adequate nutrition, education and health care.

This thesis has described the Biomarkers of Social Disadvantage Study. In a relatively small sample of healthy middle-aged adults in an affluent, small, planned, Australian city, with universal access to high-quality health care there are demonstrable socioeconomic gradients in psychosocial stressors and assets and in biological parameters that may be antecedent to ill-health. The results presented suggest complex pathways between facets of socioeconomic status, psychosocial factors and physiological changes, but particularly highlight the pervasive and fine-grained nature of socioeconomic health gradients that are present even in a group of healthy, mainly middle class, adults.
References


486. MacKinnon, D.P., *Statistical Mediation.*, RIPL.


Appendix A

The stress response and its effect on body systems

The neuroendocrine response to stress primarily involves two major body systems, the autonomic nervous system (particularly the sympathetic branch) and the hypothalamic pituitary axis.

A.1 Physiological stress pathways

A.1.1 The Autonomic Nervous System

The autonomic nervous system (ANS) consists of two limbs, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PNS). The SNS is primarily associated with conferring an adaptive advantage during stressful situations, mobilizing energy, heightening awareness and preparing for action. The PNS primarily opposes the actions of the SNS allowing tight control of body systems innervated by both.

The sympatho-adrenal medullary system (SAM) consists of the sympathetic nerves and the adrenal medulla. Its actions are mediated by noradrenaline and adrenaline, the latter being released from the adrenal medulla on stimulation by sympathetic nerves. Adrenaline is the circulating hormone of the SAM, while noradrenaline is the neurotransmitter of the postganglionic sympathetic nerve endings [235]. The effects of the SNS are induced promptly but dissipate rapidly.
A.1.2 The Hypothalamic Pituitary Adrenal Axis

The hypothalamus secretes a number of neuropeptides that act on the anterior pituitary gland to stimulate the release of pituitary hormones. Thus, corticotrophin releasing hormone (CRH) stimulates the release of adrenocorticotrophin hormone (ACTH), growth hormone releasing hormone (GHRH) stimulates the release of growth hormone (GH), thyrotrophin releasing hormone (TRH) stimulates the release of thyroid stimulating hormone (TSH) and gonadotrophin releasing hormone (GnRH) stimulates the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). In addition, dopamine inhibits the release of prolactin from the anterior pituitary, and arginine vasopressin (AVP) and oxytocin are produced in neurons of the hypothalamus and stored in the posterior pituitary. Each of these hormones has specific effects on target tissues in the periphery. Of particular interest in the stress response, ACTH stimulates the release of glucocorticoids, principally cortisol, from the adrenal cortex. Cortisol has wide ranging effects on metabolism, the immune system and the brain. These are dealt with in greater detail later in this section.

A.2 The neuroendocrine response to stress

The response to a stressor has both central and peripheral elements. The central components are located in the hypothalamus and brainstem and include:

1. The parvocellular neurons in the paraventricular nuclei of the hypothalamus, producing CRH;
2. Noradrenergic cell groups in the medulla and pons, including the locus coerulus (LC-NE);
3. The arginine vasopressin neurons of the paraventricular nuclei of the hypothalamus;
4. The CRH-producing neurons of the paragigantocellular and parabranchial nuclei of the medulla and the locus coeruleus.

Peripheral components include

1. The peripheral limbs of the HPA axis,
2. The efferent SAM,
3. Components of the PNS,
4. The immune system and the system of cytokines that provide the interface between the nervous, neuroendocrine and immune systems [595].

The central and peripheral components of the neuroendocrine stress response are shown diagrammatically in Figure A.1.

Central components:
The early signs of activation of the stress response are of activation of the sympathetic nervous system, controlled by the LC-NE, in turn stimulated by CRH released from various sites. Importantly, the LC-NE also contains CRH-producing neurons, allowing communication between the immediate neural response and the slower endocrine response. Indeed, the central CRH and LC-NE/autonomic systems function as components in a positive, reverberatory, feedback loop, in which activation of one system appears to activate the other [162].
Figure A.1: The Neuroendocrine stress response  Adapted from [158]
The LC-NE produces most of the noradrenaline in the brain and this serves as the alarm system for the brain, activating the amygdala, which is the principal brain locus for fear-related behaviours. In addition, noradrenaline promotes the long-term storage of aversively charged memories in the hippocampus [158].

CRH is released from a number of brain sites including the paraventricular nucleus of the hypothalamus and the amygdala. AVP, released from the posterior pituitary, is synergistic with CRH for the release of ACTH from the anterior pituitary. In addition, CRH and AVP have a reciprocal positive interaction, with each stimulating secretion of the other. This means that CRH and AVP continuously upregulate their secretion, allowing large amounts of ACTH to be produced. ACTH shares a common precursor (pro-opiomelanocortin, POMC) with α-melanocyte-stimulating hormone and β-endorphin, so that during the acute stress response there may be increased secretion of β-endorphin, which may, in turn stimulate the adrenal medulla to produce adrenaline. In association with increased release of CRH from the hypothalamus there is decreased release of GnRH (and thus decreased release of pituitary gonadotrophins) and decreased production of neuropeptide Y (NPY, important in stimulating appetite). ACTH acts on the adrenal gland to produce glucocorticoids (from the zona fasciculata) and adrenal androgens (from the zona reticularis).

There is a tight linkage between the immune systems and the HPA axis and immune mediators such as interleukins 1 and 6 (IL-1, IL-6) are also potent stimulators of ACTH secretion.
There are multiple inhibitory and excitatory connections within the brain stem and the cortex. The mesocorticolimbic system is activated by the stress system and has direct connections to the LC-NE and via the amygdala to the hypothalamus. The mesocortical component is involved in anticipatory phenomena and cognitive functions, while the mesolimbic component plays a principal role in motivational/reinforcement/reward phenomena. The hippocampus exerts an inhibitory influence on the activity of the LC-NE, the amygdala and the paraventricular nucleus of the hypothalamus. The amygdala-hippocampus complex is activated during stress by ascending neurons of the SNS, by glucocorticoids (GCs) and by inner emotional stressors such as fear [159]. Activation of the amygdala is important for retrieval and emotional analysis of relevant information for any given stressor. In response to emotional stressors, the amygdala can stimulate the hypothalamus, the SAM and the mesocorticolimbic system. The hippocampus exerts tonic and inhibitory effects on the activity of the amygdala and the HPA [171]. It has important functions in the memory of context, particularly where events have a strong emotional bias and allows retrieval and emotional analysis of memories associated with the stressor [172].

The stress response, in turn, facilitates the memory of events associated with strong emotions, mediated by adrenaline and GCs [157]. However, there may be stress-induced hippocampal dysfunction and memory impairment. Increased glucocorticoid secretion in the acute stress response suppresses the mechanisms in the hippocampus and temporal lobe that subserve short-term memory [165]. These effects are reversible and relatively short-lived. However, repeated stressor exposure may cause the atrophy of hippocampal neurons through a mechanism involving both cortisol and various neurotransmitters.
released during and after stress. While this atrophy is reversible if the stress is short lived, stress lasting many months or years can kill hippocampal neurons with consequent memory impairment [157, 596].

Several other brain stem nuclei (Barrington nucleus, nucleus tractus solitarius, dorsal motor vagal nucleus) act via the PNS to mediate the gut responses to stress [158].

The central components act both to activate the peripheral elements of the stress response and to facilitate the neural pathways that result in enhanced arousal, alertness, vigilance, cognition, focused attention and appropriate aggression [171].

Peripheral components:
Activation of the LC-NE and of the SNS causes release of adrenaline from the adrenal medulla and stimulation of sympathetic effects on target tissues. Peripheral changes include increased cardiovascular tone (with consequent increases in blood pressure and heart rate), increased thermogenesis, increased respiratory rate and dilatation of the pupils. These effects occur within seconds and are the basis of the fight/flight stress response.

Cortisol is released over minutes to hours and exerts its effect through widely distributed intracellular receptors. It has a permissive effect on secretion of adrenaline. It causes an increase in blood glucose by acting as an insulin antagonist, as well as by stimulating gluconeogenesis, to ensure the maximum availability of essential nutrients (e.g. oxygen and glucose) to the central nervous system and body sites such as muscles that may be
required to deal with a threat. There is an increase in protein breakdown, and lipolysis. Glucocorticoids cause an elevation of circulating polymorphonuclear leucocytes by increasing the release of mature cells from the bone marrow and inhibiting their loss through the capillaries as part of the acute inflammatory process. There is depletion of circulating eosinophils and T cells by redistribution from the circulation into other compartments. Cortisol inhibits several transcription factors (e.g. NF-κβ), which are involved in the transcription of several genes controlling the function and growth of nonimmune and immune cells. There appears to be an inverted U-shaped response of neuronal function to cortisol levels, with low concentrations maintaining, moderate concentrations promoting and high concentrations impairing function [596].

The stress response is tightly controlled by negative feedback loops. Cortisol produced by the adrenal cortex inhibits the secretion of ACTH and CRH, as well as acting on the hippocampus and the frontal cortex. While much is known about the stress response, the exact mechanism by which the response is turned on and off is still unclear.

### A.3 Effects on target systems

The stress response results in release of adrenaline and cortisol from the adrenal gland, increased activity of the SNS and inhibition of the release of a number of hormones controlling vegetative functions, e.g. growth hormone and the sex steroids. These neurohumoral products of the stress response have wide ranging effects on all body systems. Some of these effects are briefly reviewed below, as an introduction to the wide-ranging effects of stressor exposure on the body.
A.3.1 Gastrointestinal tract

Inhibition of the PNS as well as release of CRH from stress responsive enteric neurons may be responsible for the effects of stressors on the gastrointestinal system. Such effects range from diarrhea (acute stress) to gastric mucosal disruption and bleeding (stress ulcer).

A.3.2 Reproductive system

The reproductive system is inhibited at all levels by various components of the HPA axis. Both CRH and glucocorticoids suppress GnRH, inhibiting the release of LH and FSH from the anterior pituitary. GCs act on target tissues of sex steroids making them resistant to these hormones. Suppression of gonadal function is a common feature of those under severe physical stress, such as ballet dancers, gymnasts and highly trained runners [158].

A.3.3 Growth

GHRH is suppressed by CRH, leading to suppression of GH secretion from the anterior pituitary. In addition, GCs inhibit the effects of other growth factors (including insulin like growth factor 1, IGF-1) on their target tissues [158]. Prolonged activation of the stress response is associated with inhibition of growth, such as that seen in psychosocial short stature in children and adolescents with severe emotional deprivation and/or physical or psychological abuse [159].

A.3.4 Metabolic system

The metabolic system is primarily concerned with the maintenance of body tissues and energy balance in the body – the availability of energy as required (in the form of glucose), the storage of excess energy (as fat and glycogen) and the maintenance of tissue
protein. The liver is a particularly important organ for the maintenance of metabolic homeostasis. Figure A.2 outlines stress effects on the metabolic system.

**Carbohydrate metabolism**

Maintenance of blood sugar within relatively narrow limits is achieved by a combination of glycogenesis (formation of glycogen), glycogenolysis (glycogen breakdown), glycolysis (the breakdown of glucose) and gluconeogenesis (the formation of glucose) in the liver. These actions are under the control of a number of hormones, including:

1. Insulin: released from the pancreas in response to elevations of blood glucose, it increases glucose uptake by tissues, slows glycogenolysis and inhibits gluconeogenesis.

2. Glucagon: also released from the pancreas, it is antagonistic to the actions of insulin. It stimulates glycogenolysis and gluconeogenesis and the release of glucose back into the circulation.

3. Growth hormone: stimulates the growth of bones and cartilage via insulin-like growth factor (IGF-1) that is produced in the liver under GH stimulation, as well as facilitating the release of insulin. IGF-1 has some insulin like action. GH may also have a direct effect as an insulin antagonist, opposing the effects of insulin on glucose uptake and fatty acid release.

4. Adrenaline: increases the rate of glycogenolysis in the liver and skeletal muscles and the release of glucose into the blood.

5. Cortisol: stimulates gluconeogenesis from proteins and fat to increase blood sugar.
Figure A2: Carbohydrate and Fat Metabolism
Activation of both the SAM and the HPA axis results in acute increases in blood glucose, providing energy, while inhibiting vegetative functions such as muscle and bone anabolism, lipogenesis and reproduction.

**Fat metabolism**

Fat forms the body’s store of energy. Fat deposition is a balance between lipogenesis (fat formation) and lipolysis (fat breakdown). It is a complex process controlled by neuropeptide Y, leptin, the SAM pathways and the HPA axis. Leptin (secreted by adipocytes under the control of TNF-α, and IL-6 [257]) and neuropeptide Y have opposite effects on the hypothalamus inducing satiation or appetite respectively [163, 597, 598]. In addition, leptin stimulates the LC-NE system and inhibits hypothalamic CRH production, while NPY inhibits the LC-NE system and stimulates hypothalamic CRH production. Insulin promotes lipogenesis, while growth hormone and the sex hormones stimulate lipolysis. Adrenaline and noradrenaline stimulate lipolysis by stimulating hormone sensitive lipase (HSL), while cortisol inhibits the effects of insulin on skeletal muscle but potentiates its effects on adipose tissue, enhancing lipogenesis [599]. TNF-α, IL-1 and IL-6 inhibit lipogenesis and stimulate lipolysis, while IFN-γ enhances hepatic fatty acid synthesis [600].

**A.3.5 Immune system**

The human immune system is responsible for the surveillance and management of breaches of the physical protective barriers of the body, the skin and mucous membranes. The cells and molecules of the immune system combine to attack and destroy potentially invasive microbes.
Stress has long been thought to weaken the immune system, enhancing susceptibility to infectious disease. It has proven more difficult to demonstrate this conclusively in humans. The difficulty lies in the choice of immune marker, the measurement of stressor exposure and the confounding effect of psychosocial factors.

That there is communication between the HPA axis, the SMA system and the immune system is clear. The communication is bi-directional, mediated by cytokines, glucocorticoids and other hormones.

Each immune response generally has two fundamentally different elements (outlined in Figure A.3), working together:

• a primitive innate non-specific response based on phagocytic cells, complement, cytokines and natural killer cells that occurs in the same way no matter how many times the pathogen is encountered
• an adaptive or acquired specific response based on immunological memory via T cells and B cells, that responds with increasing efficiency with each repeated exposure to the pathogen.

In the acute inflammatory response to an invading pathogen, complement is activated through the classic, alternative or lectin pathways. The ensuing cascade results in the release of various elements of complement. These enhance the attraction of phagocytic cells to the site, as well as stimulating phagocytosis of the pathogen and the release of inflammatory mediators such as histamine, which in turn cause increases in local vascular permeability and further attraction of neutrophils. This is the primitive innate immune response.
The acquired immune response is based on lymphocytes and comprises antigen-specific B and T cells and antigen-presenting cells. Both T cells and B cells derive from pluripotent stem cells present in the bone marrow and fetal liver. Potential T cells migrate to the thymus and undergo maturation and differentiation there. Potential B cells develop in the bone marrow and become concentrated in lymph nodes and the spleen as well as circulating in blood and lymph. In response to antigens, naive lymphocytes may develop into T or B memory cells, cytotoxic T cells, Helper T cells (T\textsubscript{H} cells) or antibody secreting plasma cells.

T cells can be characterized by the presence of different cell surface molecules each of which has been assigned a “cluster of differentiation” or CD, number on the cell surface. Thus there are CD1, CD2, CD3 molecules, but T cells bearing the CD4 and CD8 molecules appear to be of particular importance. CD8 T cells are usually cytotoxic and kill infected cells, as well as producing cytokines such as tumour necrosis factor alpha (TNF-\textalpha) and interferon gamma (IFN-\textgamma). CD4 cells usually act as helper T cells and the CD4 population can be divided into T\textsubscript{H}1 (Type 1 helper) cells and T\textsubscript{H}2 (Type 2 helper) cells. T\textsubscript{H}2 helper cells secrete a variety of cytokines (including interleukins (IL) 4, 5, 6 and 10) that help B cells secrete protective antibodies. Most antigens are unable to
Figure A.3 The immune system (from [337])

Humoral (antibody-mediated) immune response

Antigen (1st exposure)

Engulfed by

Macrophage

Free antigens activate

Antigen-presenting cell

Becomes

Antigens displayed directly by infected cells activate

B cell

Stimulates

Helper T cell

Stimulates

Cytotoxic T cell

Helper T cell

Stimulates

Antigen (2nd exposure)

Stimulates

Memory

Plasma cells

Stimulates

Memory T cells

Stimulates

Active cytotoxic T cells

Gives rise to

Memory T cells

Gives rise to

Defend against extracellular pathogens by binding to antigens and making them easier targets for phagocytosis and complement

Defend against intracellular pathogens and cancer by binding to and lysing the infected cells or cancer cells
stimulate B cells without help from CD4 T helper cells. T_{H1} helper cells produce other cytokines, including IFN-γ and IL-2 that are involved in the activation of macrophages and cytotoxic T cells. B cells and macrophages produce IL-12, which is involved in the proliferation of natural killer cells, IFN-γ production and promotion of cell-mediated immune functions.

During the stress response, the activated SNS exerts systemic effects on immune organs by inducing the secretion of IL-6 in the systemic circulation. IL-6, in turn, stimulates GC secretion. Catecholamines inhibit IL-12 secretion from B cells and stimulate IL-10 secretion from T_{H2} helper T cells via β-adrenergic receptors. The net result of the combined effects of GCs and catecholamines is the suppression of innate and cellular immunity and stimulation of humoral immunity. Thus stress-related immunosuppression involves mainly the innate and cellular immune responses, facilitating diseases related to deficiency of this type immune response, such as the common cold, tuberculosis and certain tumours.

A.3.6 Haemostatic system

The acute stress response, as well as the coagulation cascade and the acute phase response, form a tightly interwoven matrix for the reaction, protection and restoration to health of the challenged individual. While the acute stress response is initiated by a perceived threat to homeostasis, the coagulation cascade is initiated within seconds by trauma or disease that disrupts the vascular endothelial lining and the acute phase response is initiated by activation of tissue macrophages and monocytes by bacterial by-products or mediators.
released from aggregation-induced platelet activation in the course of the coagulation cascade. The matrix is designed to protect an individual from typical evolutionary challenges – primarily physical. If an injury is sustained, the coagulation cascade and the acute phase response immediately act to diminish the extent of damage and ultimately to restore homeostasis.

**The coagulation cascade** [186]

Disruption of the vascular endothelium results in exposure of subendothelial connective tissue. Platelets adhere to this tissue and undergo release of preformed granules, producing ADP, Factor Va, platelet-derived growth factor (PDGF), fibronectin, thromoxane A2 and heparinase. Released ADP initiates changes in the conformation of platelet membrane glycoproteins, such that they bind circulating fibrinogen. In this way, the initially adherent platelets bind fibrinogen, which in turn binds further platelets to form the initial haemostatic platelet plug. This process takes only seconds. Released PDGF stimulates the growth and migration of fibroblasts and smooth muscle cells within the vessel wall to begin the definitive repair of the blood vessel. Simultaneous with the formation of the primary platelet plug, a secondary haemostatic system is initiated, resulting over several minutes in the formation of a firmer, definitive clot. The coagulation cascade involves the conversion of small amounts of a series of inactive coagulation factors to active factors with the formation of a succession of surface-bound complexes. The penultimate step is the conversion of prothrombin to thrombin in the presence of activated factor V (Factor Va), calcium and phospholipid on the surface of the activated platelets. Thrombin converts a small portion of circulating fibrinogen to fibrin, by lysis of A and B fibrinopeptides from
the alpha and beta chains of fibrinogen. The resulting fibrin monomer undergoes structural change to become cross-linked fibrin, the basis of the definitive clot.

As well as providing haemostasis, the clot forms a basis on which fibroblasts and epithelial cells can adhere and promote wound healing [601]. Formation of fibrin, in turn, enhances the rate of activation of plasminogen by plasminogen activators (tissue-type plasminogen activator (tPA) and urokinase-type plasminogen activator (u-PA)) resulting in the formation of plasmin, which acts to breakdown fibrin into fibrin degradation products (FDPs) [184]. Thus, as soon as a thrombus is formed, the fibrinolytic system is activated to control the extent of clot formation. The formation of fibrin from fibrinogen and its breakdown by plasmin is a dynamic process designed to provide the essential “plug” in a disrupted vessel (and subsequent tissue repair processes) while ensuring that the clot remains localized to the site of injury.

While the coagulation cascade is narrowly focused on restoring vascular integrity, the acute phase response has a wider role in restoring stability following a challenge – to prevent ongoing tissue damage, isolate and destroy infective organisms and activate repair processes. The acute phase response involves the immune system, the haemostatic system and the systems involved in the stress response (HPA, SAM) to provide a coordinated response.
**The acute phase response** [181]

Mast cell degranulation and aggregation-induced platelet activation result in the release of mediators that are chemotactic for tissue macrophages and blood monocytes. Activated macrophages and monocytes release a number of cytokines, particularly IL-1 and TNF-α, which, in turn act on stromal cells (including fibroblasts and endothelial cells) to cause the release of a secondary wave of cytokines including IL-8 (chemotactic for neutrophils), monocyte chemoattractant protein (MCP, chemotactic for monocytes), intercellular adhesion molecule (ICAM, promotes transendothelial migration of leukocytes) and interleukin-6 (IL-6). While many of the cytokines released (including those above) are involved in the attraction of leukocytes to the site of injury, and their migration through the vessel wall, IL-6 is a potent stimulator of the HPA axis and acts on hepatocytes to increase the production of most of the acute phase proteins (APPs). It is a primary activator of type 2 APPs – fibrinogen, haptoglobin and α1-antitrypsin. Production of other acute phase proteins, such as C-reactive protein, complement component C3, and α1-acid glycoprotein is primarily mediated by IL-1 type cytokines. Fibrinogen and the other acute phase proteins have important roles in enhancing the inflammatory and immune responses to challenge.

Glucocorticoids enhance the effect of IL-1 and IL-6 on acute phase protein production, but inhibit the production of IL-6. The acute phase response is also regulated by naturally occurring antagonists such as IL-1 receptor antagonist (IL-1RA) and soluble TNF receptor and other cytokines including IL-4 and IL-10. Once activated, the acute phase response
subsides over 24-48 hours as a result of inhibition of release of IL-1 and IL-6 by cortisol and antagonistic cytokines.

The acute stress response is activated in a similar way whether the stressor be physical or psychological, but the coagulation cascade is not activated unless there is a breach of the vascular endothelium. However, the acute phase response may be activated in both physical and psychological stress under the effect of IL-6 and GCs. Acute psychological stress appears to increase levels of IL-6 and TNF-α and cortisol enhances the effects of IL-1 and IL-6 type cytokines on the hepatic synthesis of acute phase proteins, as well as inhibiting release of IL-6 in a negative feedback control loop.

The physiological result of the acute phase response is four-fold:

1. release of mediators that cause leukocytes to be drawn to the site of injury;
2. altered vessel permeability allowing the escape into tissues of plasma constituents;
3. release of pain mediators such as bradykinin at the site of injury;
4. release of acute phase proteins, fibrinogen, haptoglobin, C-reactive protein etc.

While these outcomes are protective following a physical threat and subsequent injury, such a response to psychological stress is inappropriate. Elevated levels of IL-6, IL-1, TNF-α as well as fibrinogen and C-reactive protein are not required for the individual to recover from psychological stress and may be disease-inducing.
### Table A.1 Physiologic/somatic consequences of chronic stress system activation/target tissue effects [583]

<table>
<thead>
<tr>
<th>HPA axis cortisol</th>
<th>Locus coerulus/noradrenaline catecholeamines, IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS effects</td>
<td></td>
</tr>
<tr>
<td>+ Hippocampal negative feedback impairment</td>
<td></td>
</tr>
<tr>
<td>+ Potentiation of amygdala actions/fear</td>
<td>+ (Catecholeamines)</td>
</tr>
<tr>
<td>+ Mesocorticollimbic dopaminergic system dysfunction</td>
<td></td>
</tr>
<tr>
<td>- Leptin actions</td>
<td></td>
</tr>
<tr>
<td>- GH/IGF-1</td>
<td></td>
</tr>
<tr>
<td>- LH/T/E₂</td>
<td></td>
</tr>
<tr>
<td>- TSH/T₃</td>
<td>- (IL-6)</td>
</tr>
<tr>
<td>Increased blood pressure</td>
<td></td>
</tr>
<tr>
<td>+ Vasoconstrictor systems</td>
<td></td>
</tr>
<tr>
<td>Catecholeamines/angiotensin 2</td>
<td>+ (Catecholeamines)</td>
</tr>
<tr>
<td>Arginine-vasopressin/endothelin</td>
<td></td>
</tr>
<tr>
<td>- Vasodilatory systems</td>
<td></td>
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<tr>
<td>Kallikrein/prostacyclcin</td>
<td></td>
</tr>
<tr>
<td>Nitric oxide synthase/inflammatory cytokines</td>
<td></td>
</tr>
<tr>
<td>+ Salt retention</td>
<td></td>
</tr>
<tr>
<td>- Renin substrate</td>
<td>+ Renin (catecholeamines)</td>
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<tr>
<td>Visceral fat syndrome</td>
<td></td>
</tr>
<tr>
<td>Insulin resistance</td>
<td></td>
</tr>
<tr>
<td>+ Gluconeogenesis</td>
<td>+ (Catecholeamines)</td>
</tr>
<tr>
<td>- Peripheral glucose disposal</td>
<td></td>
</tr>
<tr>
<td>+ Insulin concentrations</td>
<td></td>
</tr>
<tr>
<td>+ Visceral fat cell growth/function</td>
<td></td>
</tr>
<tr>
<td>+ Carbohydrate intolerance</td>
<td></td>
</tr>
<tr>
<td>+ Cholesterol, LDL, small dense LDL, FFA, triglycerides</td>
<td></td>
</tr>
<tr>
<td>- HDL</td>
<td></td>
</tr>
<tr>
<td>+ Coagulation processes</td>
<td>+ (IL-6)</td>
</tr>
<tr>
<td>- Thrombolytic processes</td>
<td></td>
</tr>
<tr>
<td>- Insulin-induced vasodilation causing hypertension</td>
<td></td>
</tr>
<tr>
<td>+ Impaired insulin secretion</td>
<td></td>
</tr>
<tr>
<td>Atherosclerosis/cardiovascular diseases</td>
<td>+ (IL-6 proinflammatory activity)</td>
</tr>
<tr>
<td>Low bone mineral density/osteoporosis</td>
<td></td>
</tr>
<tr>
<td>- Osteoblastic activity</td>
<td>+ Osteoclastic activity (IL-6)</td>
</tr>
<tr>
<td>Immune dysfunction</td>
<td></td>
</tr>
<tr>
<td>- IL-12, TH₁</td>
<td>+ IL-4, IL-10, IL-13, TH₂</td>
</tr>
</tbody>
</table>

+, stimulation; -, inhibition; CNS, central nervous system; HPA axis, hypothalamic-pituitary-adrenal axis; GH/IGF-1, Growth hormone/IGF-1; LH/T/E₂, luteinizing hormone/testosterone/oestradiol; TSH/T₃, thyroid stimulating hormone/triiodothyronine; LDL, low density lipoprotein; FFA, free fatty acid; HDL, high density lipoprotein; IL, interleukin; TH₁, T helper 1; TH₂, T helper 2.
Table A.1 presents a summary of the effects of chronic activation of the stress response, on body systems.

This overview of bodily systems involved in the acute stress response forms a background to the choice of biological markers, outlined in Chapter 3, part two.
Appendix B

Study Questionnaires

THE AUSTRALIAN NATIONAL UNIVERSITY

National Centre for Epidemiology and Population Health
& The Centre for Mental Health Research

Biomarkers of Stress Study

Confidentiality
The information provided by you in this questionnaire will be treated in the strictest confidence. Information will be stored by ID number only. Names are stored separately from all other information that has been provided.

Completing the questionnaire
Please complete all questions. Work quickly, not spending too much time on any one item.
Don’t worry about being “consistent” in your answers. Respond to each question as if it was the only one.

When completed please seal the questionnaire in the envelope provided. I will collect this questionnaire at the time of the interview.

Thank you for taking part in this project.
Question 1

What is the gross income (including pensions and allowances) that you usually receive each week from all sources? If this has recently changed please mark the typical weekly income over the last year or so.

Mark one box only for personal income and one box for total household income.

Count all income including: family payment
Additional family payment
Rental assistance
Pensions
Unemployment benefits
Student allowance
Maintenance (child support)
Worker’s compensation
Superannuation
Wages
Salary
Overtime
Commissions and bonuses
Interest received
Dividends
Rents received
(less expenses of operation)
Business or farm income
(less expenses of operation)

Do not deduct: tax
Superannuation
Health insurance

Please note:
For total household income please include the income of all persons who you feel are part of your household – yourself, your partner, children, others who share food and essentials for living in your dwelling.
Personal income

- $2000 or more per week
  ($104,000 or more per year)
- $1500 - $1999 per week
  ($78,000 - $103,948 per year)
- $1000 - $1499 per week
  ($52,000 - $77,999 per year)
- $800 - $999 per week
  ($41,600 - $51,999 per year)
- $700 - $799 per week
  ($36,400 - $41,599 per year)
- $600 - $699 per week
  ($31,200 - $36,399 per year)
- $500 - $599 per week
  ($26,000 - $31,199 per year)
- $400 - $499 per week
  ($20,800 - $25,999 per year)
- $300 - $399 per week
  ($15,600 - $20,799 per year)
- $200 - $299 per week
  ($10,400 - $15,599 per year)
- $160 – $199 per week
  ($8,320 - $10,399 per year)
- $120 - $159 per week
  ($6,240 - $8,319 per year)
- $80 - $119 per week
  ($4,160 - $6,239 per year)
- $40 - $79 per week
  ($2,080 - $4,159 per year)
- $1 - $39 per week
  ($1 - $2,079 per year)
- Nil income
- Negative income

Total Household income (including your own income)

- $3500 or more per week
  ($182,000 per year)
- $3000 - $3499 per week
  ($156,000 - $181,948 per year)
- $2500 - $2999 per week
  ($130,000 - $155,948 per year)
- $2000 - $2499 per week
  ($104,000 - $129,948 per year)
- $1750 - $1999 per week
  ($91,000 - $103,948 per year)
- $1500 - $1749 per week
  ($78,000 - $90,948 per year)
- $1250 - $1499 per week
  ($65,000 - $77,948 per year)
- $1000 - $1249 per week
  ($52,000 - $64,948 per year)
- $900 - $999 per week
  ($46,800 - $51,948 per year)
- $800 - $899 per week
  ($41,600 - 46,748 per year)
- $700 - $799 per week
  ($36,400 - $41,599 per year)
- $600 - $699 per week
  ($31,200 - $36,399 per year)
- $500 - $599 per week
  ($26,000 - $31,199 per year)
- $400 - $499 per week
  ($20,800 - $25,999 per year)
- $300 - $399 per week
  ($15,600 - $20,799 per year)
- $200 - $299 per week
  ($10,400 - $15,599 per year)
- $100 - $199 per week
  ($5,200 - $10,348)
- Less than $100 per week
  ($5,200 per year)
- Nil income / negative income
2. What is your current relationship status?
   1. Married
   2. De facto
   3. Separated
   4. Divorced
   5. Widowed
   6. Never married

3. How long is it since your last marriage or de facto relationship ended?
   _____ years    _____ months

4. Have you changed jobs in the last six months?
   ☐ Yes    ☐ No (go to Q7)

5. Would you say you have more or less control of your work in the new job?
   ☐ More    ☐ Less    ☐ Same

6. Would you say there are more or less demands on you in the new job?
   ☐ More    ☐ Less    ☐ Same

7. Are you taking any medications (including oral contraceptives, over the counter preparations, prescription medications, herbal remedies, homeopathic remedies etc).
   ☐ Yes    ☐ No
   If ‘Yes” please list medication and what you use this medication for :
   Medication                          Used for:
   ___________________________________
   ___________________________________
   ___________________________________
   ___________________________________
   ___________________________________

8. Do you currently smoke?
   ☐ Yes    ☐ No

9. How many cigarettes do you usually smoke in one week?    ________

10. Have any of the following things happened to you during the last six months?
   a) You yourself suffered a serious illness, injury or an assault.
      ☐ Yes    ☐ No
   b) A serious illness, injury or assault happened to a close relative.
      ☐ Yes    ☐ No
   c) Your parent, child or partner died.
      ☐ Yes    ☐ No
   d) A close family friend or another relative (aunt, cousin, grandparent) died.
      ☐ Yes    ☐ No
   e) You broke off a steady relationship.
      ☐ Yes    ☐ No
   f) You had a serious problem with a close friend, neighbour or relative.
      ☐ Yes    ☐ No
   g) You had a crisis or serious disappointment in your work or career.
      ☐ Yes    ☐ No
   h) You thought you would soon lose your job.
      ☐ Yes    ☐ No
   i) You became unemployed or you were seeking work unsuccessfully for more than one month.
      ☐ Yes    ☐ No
   j) You were sacked from your job.
      ☐ Yes    ☐ No
k) You had a major financial crisis.
   - Yes  - No

l) You had problems with the police and a court appearance.
   - Yes  - No

m) Something you valued was lost or stolen.
   - Yes  - No

11. What do you consider to be the major stresses in your life in the last six months?

12. How would you rate your average stress level over the last six months?
   - High  - Medium  - Low

By partner we mean spouse or de facto partner.

13. What is your partner’s job title? If more than one job, record title of main job.
(Please include level/rank)

14. What are his/her main duties or activities?

15. Is he/she
   - Employed by a government agency
   - Employed by a profit-making organization
   - Self-employed. Please state number of employees
   - Other

16. Which of the following best describes the position he/she holds within the business or organisation?
   - Managerial position
   - Supervisory position
   - Non-management position

17. Have any of the following happened in the last six months?
   a) Your partner thought he/she would soon lose his/her job?
      - Yes  - No
   b) Your partner had a crisis or serious disappointment in his/her work or career.
      - Yes  - No

18. About how many close friends and close relatives do you have (people you feel at ease with and can talk to about what is on your mind)?

Write in the number of close friends and close relatives.

19. How many registered motor vehicles are owned and/or used by members of this household that are usually garaged or parked near your dwelling? (Exclude motor bikes, motor scooters, and tractors. Include company vehicles kept at home)

   0  - None
   1  - One motor vehicle
   2  - Two motor vehicles
   3  - Three motor vehicles
   4  - Four or more motor vehicles
   5  - Not Applicable (e.g. no driver’s licence)
Now I want to ask you some questions about your childhood.

When you were age 12, what was the highest educational achievement of:

20. Your mother

1  ○ No schooling
2  ○ Some primary schooling, but did not complete
3  ○ Completed primary school
4  ○ Left school before age 15, no qualifications later
5  ○ Completed high school, no qualifications later
6  ○ Trade certificate, diploma or equivalent
7  ○ University degree

21. Your father

1  ○ No schooling
2  ○ Some primary schooling, but did not complete
3  ○ Completed primary school
4  ○ Left school before age 15, no qualifications later
5  ○ Completed high school, no qualifications later
6  ○ Trade certificate, diploma or equivalent
7  ○ University degree

At age 12, what were your parents’ occupations? (Title and brief description of job)

22. Mother:
   Job Title (include rank/level)
   ______________________________________________________
   Job description:
   ______________________________________________________

23. Father:
   Job Title (include rank/level):
   ______________________________________________________
   Job description:
   ______________________________________________________

This is the end of the section about your childhood.

24. How would you describe your accommodation? (Please mark ONE circle only)

1  ○ Rented house
2  ○ Rented flat/unit/apartment/townhouse
3  ○ Own house
4  ○ Own flat/unit/apartment/townhouse
5  ○ ACT Housing Trust house
6  ○ ACT Housing Trust flat/unit/semi-detached house
7  ○ Living with relatives who are the primary occupants of a dwelling
8  ○ Other eg caravan park, mobile, please specify
   ______________________________________________________

25. These questions ask what you think about the way most people behave nowadays. Please mark the circle below each statement that is closest to your opinion.

a) Most people tell the truth when they’re sorting out a problem.

1  ○ Definitely disagree
2  ○ Disagree
3  ○ Disagree a bit
4  ○ Neither agree nor disagree
5  ○ Agree a bit
6  ○ Agree
7  ○ Definitely agree

b) Most people make agreements honestly.

1  ○ Definitely disagree
2  ○ Disagree
3  ○ Disagree a bit
4  ○ Neither agree nor disagree
5  ○ Agree a bit
6  ○ Agree
7  ○ Definitely agree

c) Most people don’t mislead others.

1  ○ Definitely disagree
2  ○ Disagree
3  ○ Disagree a bit
4  ○ Neither agree nor disagree
5  ○ Agree a bit
6  ○ Agree
7  ○ Definitely agree
d) Most people decide what they expect from each other fairly.
   1 □ Definitely disagree
   2 □ Disagree
   3 □ Disagree a bit
   4 □ Neither agree nor disagree
   5 □ Agree a bit
   6 □ Agree
   7 □ Definitely agree

Now I would like to ask some questions about how you feel about your neighbourhood.

26. Generally speaking, would you say that:
   a). Most people can be trusted or that you can't be too careful in dealing with people?
      1 □ Most people can be trusted
      2 □ Can't be too careful

   b). Most of the time people try to be helpful or are they mostly looking out for themselves?
      1 □ People try to be helpful
      2 □ People are mostly looking out for themselves

27. The following statements are about how much support you have in your community. Please read each one quickly and mark True if the statement is probably true for you, and False if it is probably false. If a statement seems neither clearly true nor clearly false, try to decide quickly which is more likely.

   a) No-one I know would throw a birthday party for me.
      □ True □ False

   c) When I feel lonely there are several people I could call and talk to.
      □ True □ False

   c) If I decide one afternoon that I would like to go to a movie that evening I could find someone to go with me.
      □ True □ False

   d) I don't often get invited to do things with others.
      □ True □ False

   e) If I wanted to go out of town (eg to the coast) for the day, I would have a hard time finding someone to go with me.
      □ True □ False

   f) If I wanted to have lunch with someone I could easily find someone to join me.
      □ True □ False

   g) I feel that I’m on the fringe in my circle of friends.
      □ True □ False

   h) There are several different people with whom I enjoy spending time.
      □ True □ False

   i) Most people I know don’t enjoy the same things that I do.
      □ True □ False

   j) I regularly meet or talk with members of my family or friends.
      □ True □ False

28. These questions ask about how often you join in things in your community. Please use the answer code to circle the number that is closest to what you do for each statement below.

   a) I eat my main meal with members of my immediate family.
      1 □ Never
      2 □ Almost never
      3 □ Sometimes
      4 □ Fairly often
      5 □ Very Often

   b) I do unpaid voluntary work or helping out for free (eg, school P&C, fund-raising walks, meals-on-wheels, cooking sausages at a fete, selling raffle tickets).
      1 □ Never
      2 □ Almost never
      3 □ Sometimes
      4 □ Fairly often
      5 □ Very Often

   c) I pay membership fees to a group that organises activities in my community (eg, sports club, choir, reading circle.)
      1 □ Never
      2 □ Almost never
      3 □ Sometimes
      4 □ Fairly often
      5 □ Very Often
d) I go to courses on subjects that interest me.
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

k) I talk to my neighbours “over the fence” or in “in the stairwell”.
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

l) I take an active part in at least one group that organises activities in my community (e.g., sports club, choir, reading circle.)
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

m) I make time to attend services at a place of worship.
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

h) My friends come over to my place or I go to theirs.
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

i) When I do unpaid voluntary work or helping out for free, I take on jobs like secretary or treasurer.
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

j) I make time to keep in touch with my friends.
   1  ○ Never
   2  ○ Almost never
   3  ○ Sometimes
   4  ○ Fairly often
   5  ○ Very Often

29. Think of this ladder as representing where people stand in their communities.

People define community in different ways; please define it in whatever way is most meaningful to you. At the top of the ladder are the people who have the highest standing in their community. At the bottom are the people who have the lowest standing in their community.

Where would you place yourself on this ladder? Please place a large "X" on the rung where you think you stand at this time in your life, relative to other people in your community.
The next questions are about how you feel about yourself.

30. How strongly do you agree or disagree with these statements? Please use the answer code to circle the number that is closest to how you feel about each statement.

<table>
<thead>
<tr>
<th>Strongly Agree</th>
<th>Agree</th>
<th>Disagree</th>
<th>Strongly Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

1. I feel that I’m a person of worth, at least on an equal with others.
   1 2 3 4

2. I feel that I have a number of good qualities.
   1 2 3 4

3. All in all, I am inclined to feel that I’m a failure.
   1 2 3 4

4. I am able to do things as well as most other people.
   1 2 3 4

5. I feel I do not have much to be proud of.
   1 2 3 4

6. I take a positive attitude toward myself.
   1 2 3 4

7. On the whole, I am satisfied with myself.
   1 2 3 4

8. I certainly feel useless at times.
   1 2 3 4

9. I wish I could have more respect for myself.
   1 2 3 4

10. At times I think I am no good at all.
    1 2 3 4

The following questions try to look at how you see life.

31. How strongly do you agree or disagree with these statements? Please use the answer code to circle the number that is closest to how you feel about each statement. Please note that this is a different code to the previous question.

<table>
<thead>
<tr>
<th>Strongly Agree</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
<th>Strongly Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

1. In uncertain times, I usually expect the best.
   1 2 3 4 5

2. It’s easy for me to relax.
   1 2 3 4 5

3. If something can go wrong for me, it will.
   1 2 3 4 5

4. I’m always optimistic about my future.
   1 2 3 4 5

5. I enjoy my friends a lot.
   1 2 3 4 5

6. It’s important for me to keep busy.
   1 2 3 4 5

7. I hardly ever expect things to go my way.
   1 2 3 4 5

8. I don’t get upset too easily.
   1 2 3 4 5

9. I rarely count on good things happening to me.
   1 2 3 4 5

10. Overall, I expect more good things to happen to me than bad.
    1 2 3 4 5
32. The following questions ask you about your feelings and thoughts during the last month. In each case, please indicate how often you felt or thought a certain way.

(a) In the last month, how often have you been upset because of something that happened unexpectedly?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(b) In the last month, how often have you felt that you were unable to control the important things in your life?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(c) In the last month, how often have you felt nervous and “stressed”?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(d) In the last month, how often have you felt confident about your ability to handle your personal problems?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(e) In the last month, how often have you felt that things were going your way?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(f) In the last month, how often have you found that you could not cope with all the things that you had to do?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(g) In the last month, how often have you been able to control irritations in your life?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(h) In the last month, how often have you felt that you were on top of things?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(i) In the last month, how often have you been angered because of things that were outside of your control?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

(j) In the last month, how often have you felt difficulties were piling up so high that you could not overcome them?
   1  Never
   2  Almost never
   3  Sometimes
   4  Fairly often
   5  Very Often

33. At the present time:

a) Are you able to afford a home suitable for (yourself/your family)?
   1  Yes
   2  No

b) Are you able to afford furniture or household equipment that needs to be replaced?
   1  Yes
   2  No

c) Are you able to afford the kind of car you need?
   1  Yes
   2  No

d) Do you have enough money for the kind of food (you/your family) should have?
   1  Yes
   2  No
e) Do you have enough money for the kind of medical care (you/your family) should have?
   1  ☐ Yes
   2  ☐ No

f) Do you have enough money for the kind of clothing (you/your family) should have?
   1  ☐ Yes
   2  ☐ No

g) Do you have enough money for the leisure activities (you/your family) want(s)?
   1  ☐ Yes
   2  ☐ No

h) Do you have any difficulty in paying your bills?
   1  ☐ A great deal of difficulty
   2  ☐ Some difficulty
   3  ☐ A little difficulty
   4  ☐ No difficulty

i) At the end of the month/fortnight do you end up with:
   1  ☐ some money left over,
   2  ☐ just enough to make ends meet,
   3  ☐ not enough money to make ends meet.

j) Do you go to the dentist for routine dental check-ups?
   1  ☐ Yes, about once a year
   2  ☐ Yes, but less than once a year
   3  ☐ Yes, but it’s a rare event
   4  ☐ No, never

k) Does your partner go to the dentist for routine dental check-ups?
   1  ☐ Yes, about once a year
   2  ☐ Yes, but less than once a year
   3  ☐ Yes, but it’s a rare event
   4  ☐ No, never

l) Do you have household contents/household effects insurance?
   1  ☐ Yes, comprehensive and to full replacement value, including all items of value.
   2  ☐ Yes, comprehensive but not quite to full replacement value of all items.
   3  ☐ Yes, partial cover
   4  ☐ No, none

34. We are interested in how people respond when they confront difficult or stressful events in their lives. There are lots of ways to try to deal with stress. This part of the questionnaire asks you to indicate what you generally do and feel, when you experience stressful events. Obviously, different events bring out somewhat different responses, but think about what you usually do when you are under a lot of stress. You should treat each item separately from every other item. There are no right or wrong answers.

Please circle one number for each question.

<table>
<thead>
<tr>
<th></th>
<th>I usually don’t do this at all</th>
<th>I usually do this a little bit</th>
<th>I usually do this a medium amount</th>
<th>I usually do this a lot</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

In response to a stressful event:

1) I turn to work or other activities to take my mind off things.
   1  2  3  4

2) I concentrate my efforts on doing something about the situation I’m in.
   1  2  3  4

3) I say to myself “this isn’t real”
   1  2  3  4

4) I use alcohol or other drugs to make myself feel better.
   1  2  3  4

5) I get emotional support from others.
   1  2  3  4

6) I give up trying to deal with it.
   1  2  3  4

7) I take action to try to make the situation better.
   1  2  3  4

8) I refuse to believe that it has happened.
   1  2  3  4

9) I say things to let my unpleasant feelings escape.
   1  2  3  4
10) I get help and advice from other people.
   1) 2) 3) 4)

11) I use alcohol or other drugs to help me get through it.
   1) 2) 3) 4)

12) I try to see it in a different light, to make it seem more positive.
    1) 2) 3) 4)

13) I criticize myself.
    1) 2) 3) 4)

14) I try to come up with a strategy about what to do.
    1) 2) 3) 4)

15) I get comfort and understanding from someone.
    1) 2) 3) 4)

16) I give up the attempt to cope.
    1) 2) 3) 4)

17) I look for something good in what is happening.
    1) 2) 3) 4)

18) I make jokes about it.
    1) 2) 3) 4)

19) I do something to think about it less, such as going to movies, watching TV, reading, daydreaming, sleeping, or shopping.
    1) 2) 3) 4)

20) I accept the reality of the fact that it has happened.
    1) 2) 3) 4)

21) I express my negative feelings.
    1) 2) 3) 4)

22) I try to find comfort in my religion or spiritual beliefs.
    1) 2) 3) 4)

23) I try to get advice or help from other people about what to do.
    1) 2) 3) 4)

24) I learn to live with it.
    1) 2) 3) 4)

25) I think hard about what steps to take.
    1) 2) 3) 4)

26) I blame myself for things that happened.
    1) 2) 3) 4)

27) I pray or meditate.
    1) 2) 3) 4)

28) I make fun of the situation.
    1) 2) 3) 4)

The next few questions ask about your situation at work. If you are not in the workforce, please go to Q 59.

35. Do you have a choice in deciding how you do your job?
    1) Often
    2) Sometimes
    3) Rarely
    4) Never

36. Do you have a choice in deciding what you do at work?
    1) Often
    2) Sometimes
    3) Rarely
    4) Never

37. Others take decisions concerning my work.
    1) Often
    2) Sometimes
    3) Rarely
    4) Never

38. I have a good deal to say in decisions about work.
    1) Often
    2) Sometimes
    3) Rarely
    4) Never
39. I have a say in my own work speed.
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

40. My working time can be flexible.
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

41. I can decide when to take a break.
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

42. I have a say in choosing with whom I work.
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

43. I have a great deal of say in planning my work environment.
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

44. Do you have to do the same thing over and over again?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

45. Does your job provide you with a variety of interesting things?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

46. Is your job boring?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

47. Do you have the possibility of learning new things through your work?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

48. Does your work demand a high level of skill or expertise?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

49. Does your job require you to take initiative?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

50. Do you have to work very fast?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

51. Do you have to work very intensively?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

52. Do you have enough time to do everything?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

53. Do different groups at work demand things from you that you think are hard to combine?
   1  O Often
   2  O Sometimes
   3  O Rarely
   4  O Never

54. How secure do you feel about your job or career future in your current workplace?
   1  O Not at all secure
   2  O Moderately secure
   3  O Secure
   4  O Extremely secure
55. If you lost your present job, how difficult do you think it would be to get another job (with the same pay and same hours)?
1  ◯ Not at all difficult
2  ◯ Moderately difficult
3  ◯ Difficult
4  ◯ Extremely difficult

56. For the work you do in your main job, how fair is the pay, benefits and conditions you receive?
1  ◯ Completely unfair
2  ◯ Somewhat unfair
3  ◯ Somewhat fair
4  ◯ Completely fair

57. How many hours do you work in a routine week (including unpaid overtime, work taken home, etc)?

_________ hours/week

58. How many working days in the last four weeks have you stayed away from your work (or school, or place of study)?

_________ days

59. During the past MONTH how often have you set aside time just to relax?
1  ◯ Not at all
2  ◯ Some or a little of the time (about once a month or more)
3  ◯ Occasionally or a moderate amount of the time (about once a week or more)
4  ◯ Often or a lot of the time (about once a day)
5  ◯ Frequently (more than once a day)

60. To what extent are you responsible for household tasks? (these include such activities as preparing meals, shopping, cleaning, washing clothes and gardening).
1  ◯ Fully responsible (100%)
2  ◯ 75% responsible
3  ◯ 50% responsible
4  ◯ 25% responsible
5  ◯ Not at all responsible (0%)

61. To what extent are you responsible for childcare in your household? (Children’s care includes activities such as making meals, organising activities, supervising homework, discipline).
1  ◯ Fully responsible (100%)
2  ◯ 75% responsible
3  ◯ 50% responsible
4  ◯ 25% responsible
5  ◯ Not at all responsible (0%)

62. To what extent are you responsible for financial management in your household? (Financial management includes paying bills, saving, planning investments or priorities in money use).
1  ◯ Fully responsible (100%)
2  ◯ 75% responsible
3  ◯ 50% responsible
4  ◯ 25% responsible
5  ◯ Not at all responsible (0%)

63. To what extent are you responsible for providing the money for your household?
1  ◯ Fully responsible (100%)
2  ◯ 75% responsible
3  ◯ 50% responsible
4  ◯ 25% responsible
5  ◯ Not at all responsible (0%)

64. How would you describe your current employment status?
1  ◯ Employed full time
2  ◯ Employed part-time, looking for full-time work
3  ◯ Employed part-time
4  ◯ Unemployed, looking for work
5  ◯ Not in the workforce
65. Think of this ladder as representing where people ‘stand’ in Australia.

At the top of the ladder are the people who are the best off – those who have the most money, the most education and the most respected jobs. At the bottom are the people who are the worst off – who have the least money, least education, and the least respected jobs or no job. The higher up you are on this ladder, the closer you are to the people at the very top; the lower you are, the closer you are to the people at the very bottom.

Where would you place yourself on this ladder?

Please place a large “X” on the rung where you think you stand at this time in your life, relative to other people in Australia.

Thank you for completing this part of the study.
Do you have any comments you would like to make about the questionnaire or the study in general?
Biomarkers of Stress Study

Part 2

Update of PATH interview

Please complete all questions, seal in the envelope provided and post to:

Dr R Lucas
NCEPH
ANU
Canberra 0200
The following questions ask for your views about your health, how you feel and how well you are able to do your usual activities on a typical day. If you are unsure about how to answer a question, please give the best answer you can.

1. Does your health now limit you in moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf?
   1  Yes – limited a lot
   2  Yes – limited a little
   3  No – not at all

2. Does your health now limit you in climbing several flights of stairs?
   1  Yes – limited a lot
   2  Yes – limited a little
   3  No – not at all

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

3. Have you accomplished less than you would like as a result of your physical health?
   ○ Yes  ○ No

4. Were you limited in the kind of work or other activities as a result of your physical health?
   ○ Yes  ○ No

During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

5. Have you accomplished less than you would like as a result of any emotional problems?
   ○ Yes  ○ No

6. Did you not do work or other activities as carefully as usual as a result of any emotional problems?
   ○ Yes  ○ No

During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

   1  ○ Not at all
   2  ○ A little bit
   3  ○ Moderately
   4  ○ Quite a bit
   5  ○ Extremely

The next few questions are about how you feel and how things have been with you during the past four weeks. For each question, please give the one answer that comes closest to the way you have been feeling.

8. How much of the time during the past 4 weeks have you felt calm and peaceful?
   1  ○ All of the time
   2  ○ Most of the time
   3  ○ A good bit of the time
   4  ○ Some of the time
   5  ○ A little of the time
   6  ○ None of the time

9. How much of the time during the past 4 weeks did you have a lot of energy?
   1  ○ All of the time
   2  ○ Most of the time
   3  ○ A good bit of the time
   4  ○ Some of the time
   5  ○ A little of the time
   6  ○ None of the time

10. How much of the time during the past 4 weeks have you felt down?
    1  ○ All of the time
    2  ○ Most of the time
    3  ○ A good bit of the time
    4  ○ Some of the time
    5  ○ A little of the time
    6  ○ None of the time
11. How much of the time during the past 4 weeks has your physical health or emotional problems interfered with your social activities (like visiting with friends, relatives, etc)?
   1. All of the time
   2. Most of the time
   3. A good bit of the time
   4. Some of the time
   5. A little of the time
   6. None of the time

The next group of questions are about your relationships with other people.

12. How often do friends make you feel cared for?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

13. How often do they express interest in how you are doing?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

14. How often do friends make too many demands on you?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

15. How often do they criticise you?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

16. How often do friends create tensions or arguments with you?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

17. How often do family members make you feel cared for?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

18. How often do family members express interest in how you are doing?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

19. How often do they make too many demands on you?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

20. How often do family members criticise you?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

21. How often do they create tensions or arguments with you?
   1. Often
   2. Sometimes
   3. Rarely
   4. Never

If you don’t have a current partner, go to Q 32

22. How much does your partner understand the way you feel about things?
   1. A lot
   2. Some
   3. A little
   4. Not at all

23. How much can you depend on your partner to be there when you really need him/her?
   1. A lot
   2. Some
   3. A little
   4. Not at all

24. How much does your partner show concern for your feelings and problems?
   1. A lot
   2. Some
   3. A little
   4. Not at all
25. How much can your trust your partner to keep promises to you?

1. A lot
2. Some
3. A little
4. Not at all

26. How much can you open up to your partner about things that are really important to you?

1. A lot
2. Some
3. A little
4. Not at all

27. How much tension is there between you and your partner?

1. A lot
2. Some
3. A little
4. Not at all

28. How often do you have an unpleasant disagreement with your partner?

1. A lot
2. Some
3. A little
4. Not at all

29. How often do things become tense when the two of you disagree?

1. A lot
2. Some
3. A little
4. Not at all

30. How often does your partner say cruel or angry things during a disagreement?

1. A lot
2. Some
3. A little
4. Not at all

31. How often do the two of you both refuse to compromise during disagreements?

1. A lot
2. Some
3. A little
4. Not at all

32. The following scale consists of a number of words that describe different feelings or emotions. Please read each item and indicate to what extent you have been feeling this way in the past month.

<table>
<thead>
<tr>
<th>Feeling</th>
<th>1: Very slightly or not at all</th>
<th>2: A little</th>
<th>3: Moderately</th>
<th>4: Quite a bit</th>
<th>5: Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disgusted</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attentive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scornful</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Irritable</td>
<td></td>
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<tr>
<td>Inspired</td>
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<td></td>
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<tr>
<td>Afraid</td>
<td></td>
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<td></td>
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<tr>
<td>Alert</td>
<td></td>
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<tr>
<td>Upset</td>
<td></td>
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</tbody>
</table>

466
Next are some specific questions about your health and how you have been feeling in the past month.

In the past month:

33. Have you felt keyed up or on edge?
   - Yes
   - No

34. Have you been worrying a lot?
   - Yes
   - No

35. Have you been irritable?
   - Yes
   - No

36. Have you had difficulty relaxing?
   - Yes
   - No

37. Have you been sleeping poorly?
   - Yes
   - No
38. Have you had headaches or neckaches?
   ○ Yes ○ No

In the past month:
39. Have you had any of the following: trembling, tingling, dizzy spells, sweating, diarrhoea, or needing to pass water more often than usual?
   ○ Yes ○ No

40. Have you been worried about your health?
   ○ Yes ○ No

41. Have you had difficulty falling asleep?
   ○ Yes ○ No

42. Have you been lacking energy?
   ○ Yes ○ No

43. Have you lost interest in things?
   ○ Yes ○ No

44. Have you lost confidence in yourself?
   ○ Yes ○ No

45. Have you felt hopeless?
   ○ Yes ○ No

46. Have you had difficulty concentrating?
   ○ Yes ○ No

47. Have you lost weight (due to poor appetite)?
   ○ Yes ○ No

48. Have you been waking early?
   ○ Yes ○ No

49. Have you felt slowed up?
   ○ Yes ○ No

50. Have you tended to feel worse in the mornings?
   ○ Yes ○ No

How strongly do you agree or disagree with the following statements?

51. There is really no way I can solve some of the problems I have.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree

52. Sometimes I feel that I’m being pushed around in life.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree

53. I have little control over the things that happen to me.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree

54. I can do just about anything I really set my mind to do.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree

55. I often feel helpless in dealing with the problems of life.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree

56. What happens to me in the future mostly depends on me.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree

57. There is little I can do to change many of the important things in my life.
   1 ○ Strongly agree
   2 ○ Agree
   3 ○ Disagree
   4 ○ Strongly disagree
People think and do many different things when they feel sad, blue or depressed. Please read each of the items below and indicate whether you never, sometimes, often or always think or do each one when you feel sad, down or depressed. Please indicate what you generally do, not what you think you should do.

58. I think about how alone I feel.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

59. I think about my feelings of fatigue and achiness.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

60. I think about how hard it is to concentrate.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

61. I think about how passive and unmotivated I feel.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

62. I think, “Why can’t I get going?”
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

63. I think about a recent situation, wishing it had gone better.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

64. I think about how sad I feel.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

65. I think about all my shortcomings, failings, faults and mistakes.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

66. I think about how I don’t feel up to doing anything.
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

67. I think, “Why can’t I do things better?”
   1 ☐ Never
   2 ☐ Sometimes
   3 ☐ Often
   4 ☐ Always

The following questions concern the way you behave, feel and act. Decide for each question whether “YES” or “NO” represents your usual way of acting or feeling. Work quickly, and don’t spend too much time over any question.

68. Does your mood often go up and down?
   ☐ Yes ☐ No

69. Do you take much notice of what people think?
   ☐ Yes ☐ No

70. Are you a talkative person?
   ☐ Yes ☐ No

71. Do you ever feel “just miserable” for no reason?
   ☐ Yes ☐ No

72. Would being in debt worry you?
   ☐ Yes ☐ No

73. Are you rather lively?
   ☐ Yes ☐ No

74. Are you an irritable person?
   ☐ Yes ☐ No

75. Would you take drugs which may have strange or dangerous effects?
   ☐ Yes ☐ No
76. Do you enjoy meeting new people?
   ☐ Yes ☐ No

77. Are your feelings easily hurt?
   ☐ Yes ☐ No

78. Do you prefer to go your own way rather than act by the rules?
   ☐ Yes ☐ No

79. Can you usually let yourself go and enjoy yourself at a lively party?
   ☐ Yes ☐ No

80. Do you often feel ‘fed-up’?
   ☐ Yes ☐ No

81. Do good manners and cleanliness matter much to you?
   ☐ Yes ☐ No

82. Do you usually take the initiative in making new friends?
   ☐ Yes ☐ No

83. Would you call yourself a nervous person?
   ☐ Yes ☐ No

84. Do you think marriage is old-fashioned and should be done away with?
   ☐ Yes ☐ No

85. Can you easily get some life into a rather dull party?
   ☐ Yes ☐ No

86. Are you a worrier?
   ☐ Yes ☐ No

87. Do you enjoy co-operating with others?
   ☐ Yes ☐ No

88. Do you tend to keep in the background on social occasions?
   ☐ Yes ☐ No

89. Does it worry you if you know there are mistakes in your work?
   ☐ Yes ☐ No

90. Would you call yourself tense or ‘highly-strung’?
    ☐ Yes ☐ No

91. Do you think people spend too much time safeguarding their future with savings and insurance?
    ☐ Yes ☐ No

92. Do you like mixing with people?
    ☐ Yes ☐ No

93. Do you worry too long after an embarrassing experience?
    ☐ Yes ☐ No

94. Do you try not to be rude to people?
    ☐ Yes ☐ No

95. Do you like plenty of bustle and excitement around you?
    ☐ Yes ☐ No

96. Do you suffer from “nerves”?
    ☐ Yes ☐ No

97. Would you like other people to be afraid of you?
    ☐ Yes ☐ No

98. Are you mostly quiet when you are with other people?
    ☐ Yes ☐ No

99. Do you often feel lonely?
    ☐ Yes ☐ No

100. Is it better to follow society’s rules than go your own way?
    ☐ Yes ☐ No

101. Do other people think of you as being very lively?
    ☐ Yes ☐ No

102. Are you often troubled about feelings of guilt?
    ☐ Yes ☐ No

103. Can you get a party going?
    ☐ Yes ☐ No
Each of the following is a statement that a person may either agree or disagree with. Indicate how much you agree or disagree with each statement.

Please be as accurate and as honest as you can be. Respond to each item as if it were the only item. That is, don’t worry about being ‘consistent’ in your responses.

<table>
<thead>
<tr>
<th>Statement</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>104. A person’s family is the most important thing in life.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>105. Even if something bad is about to happen to me, I rarely experience fear or nervousness.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>106. I go out of my way to get things I want.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>107. When I’m doing well at something, I love to keep at it.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>108. I’m always willing to try something new if I think it will be fun.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>109. How I dress is important to me.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>110. When I get something I want, I feel excited and energised.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>111. Criticism or scolding hurts me quite a bit.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>112. When I want something I usually go all-out to get it.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>113. I will often do things for no other reason than that they might be fun.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>114. It’s hard for me to find the time to do things such as get a hair cut.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>115. If I see a chance to get something I want I move on it right away.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>116. I feel pretty worried or upset when I think or know somebody is angry at me.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
<tr>
<td>117. When I see an opportunity for something I like I get excited right away.</td>
<td>1</td>
<td>Very false for me</td>
<td>Somewhat false for me</td>
<td>Very true for me</td>
</tr>
</tbody>
</table>
118. I often act on the spur of the moment.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

119. If I think something unpleasant is going to happen I usually get pretty ‘worked-up’.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

120. I often wonder why people act the way they do.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

121. When good things happen to me, it affects me strongly.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

122. I feel worried when I think I have done poorly at something important.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

123. I crave excitement and new sensations.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

124. When I go after something, I use a ‘no-holds-barred’ approach.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

125. I have very few fears compared to my friends.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

126. It would excite me to win a contest.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

127. I worry about making mistakes.
   1. Very false for me
   2. Somewhat false for me
   3. Somewhat true for me
   4. Very true for me

128. How often do you take part in sports or activities that are mildly energetic, moderately energetic or vigorous?

<table>
<thead>
<tr>
<th>Activities</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mildly energetic (eg walking, woodwork, weeding, hoeing, bicycle repair, playing pool, general housework).</td>
<td>1. 3 times a week or more</td>
<td>2. Once or twice a week</td>
<td>3. About 1-3 times a month</td>
<td>4. Never/hardly ever</td>
</tr>
<tr>
<td>Moderately energetic (eg scrubbing, polishing car, dancing, golf, cycling, decorating, lawn mowing, leisurely swimming).</td>
<td>1. 3 times a week or more</td>
<td>2. Once or twice a week</td>
<td>3. About 1-3 times a month</td>
<td>4. Never/hardly ever</td>
</tr>
<tr>
<td>Vigorous (eg running, hard swimming, tennis, squash, digging, cycle racing).</td>
<td>1. 3 times a week or more</td>
<td>2. Once or twice a week</td>
<td>3. About 1-3 times a month</td>
<td>4. Never/hardly ever</td>
</tr>
</tbody>
</table>

Please give the average number of hours per week you spend in such sports or activities.

<table>
<thead>
<tr>
<th>Mildly energetic (eg walking, weeding)</th>
<th>Hours</th>
<th>minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moderately energetic (eg dancing, cycling)</td>
<td>Hours</td>
<td>minutes</td>
</tr>
<tr>
<td>Vigorous (eg running, squash)</td>
<td>Hours</td>
<td>minutes</td>
</tr>
</tbody>
</table>
Congratulations, you have completed Part 2 of the Questionnaire. Thank you very much for your time and patience.

Please seal in the reply paid envelope and post to:

Dr R Lucas
NCEPH
ANU
Canberra 0200
Appendix C

Protocol for the collection and determination of IgA in saliva by enzyme linked immunosorbent assay.

C.1 Specimen Details

C.1.1 Specimen Collection

Saliva is collected by gentle suction using a paediatric suction set directly into a paediatric collection tube.

The saliva is immediately placed on ice and frozen at –20°C within one hour of collection.

Contamination with food or coloured substances is to be avoided.

Saliva is collected 2 hours post-prandially with no stimulation.

C.1.2 Specimen Preparation

Remove the saliva from the collection tube and store in a Nunc cryotube at –70°C.

Microfuge the sample for 5 minutes on speed 2 or centrifuge at 3000rpm for 10 minutes. The supernatant is aspirated and used directly in the assay.

C.1.3 Specimen Stability

The saliva sample can be kept for 2 years if stored at –20°C and 10 years at –70°C.

If the sample is kept at 4°C it will be stable for 1 hour. When assaying it is important to keep the sample at 4°C and return to the freezer as soon as possible.

Repeated freeze/thawing must be avoided as there is a 1-2% loss of stability each time.
C.2 Assay Method

C.2.1 Principle of Assay

Immunoglobulin class specific goat anti-human antibody adsorbed to the surface of an immunoplate acts as antigen to salivary antibody. The complex formed when standard or sample is added to the immunoplate is then bound to horseradish peroxidase-conjugated goat anti-human antibody. Tetramethylbenzidine is added to the plate as the substrate for a colour reaction. The reaction is stopped with 0.5M H$_2$SO$_4$ after incubation and the absorbance read at 450nm. A quantifiable correlation exists between the intensity of colour development and the concentration of antibody in the sample.

C.2.2 Materials

Sodium chloride

Sodium carbonate Na$_2$CO$_3$

Sodium bicarbonate NaHCO$_3$

Citric acid

Sodium hydrogen phosphate, anhydrous Na$_2$HPO$_4$

Skim Milk Powder (Diploma)

Unconjugated goat anti human IgA

HRP-conjugated anti-human IgA

Standard human IgA

Hydrogen peroxide – 30%w/v

3’,3’,5’,5’-tetramethylbenzidine (TMB)

Dimethyl sulfoxide (DMSO)

Tween 20 (Amresco 00777)
C.2.3 Equipment

Finn pipetting system – 0.5-10µl, 5-40µl, 40-200µl, 200-1000µl, 1-5ml, 1-10ml.

Pipette tips

Magnetic stirrer

Nunc Maxisorb 96 well immunoplates

Multichannel pipettes - 5-50µl, 50-300µl

Microfuge tubes

4L container for Wash Solution

Weighing trays

Measuring cylinders – 100ml, 200ml, 2L

Molecular Devices Thermomax Microplate Reader

IBM Personal Computer

Printer

Power Supply

Magnetic stirring fleas

Absorbent wash cloths

Nunc cryotubes

Paediatric suction collection sets

Suction pump

pH meter

1 litre glass bottles (6)

C.2.4 Reagents

Carbonate buffer, pH 9.6, 0.1M

Sodium carbonate

Sodium bicarbonate
PBS Tween 20, 0.05%, pH 7.2
5% skim milk in PBS
ELISA diluent; 0.1% skim milk in PBS-Tween 20.
TMB substrate buffer
Citric acid
Trisodium citrate
0.5M H₂SO₄
Unconjugated anti-IgA diluted 1:2000 in carbonate buffer
Conjugated anti-IgA diluted 1:2000 in 0.1% skim milk PBS-Tween 20, 0.05%
10 dilutions of standard human IgA in 0.1% skim milk PBS-Tween 20, 0.05%
10X PBS

C.2.5  Reagent Preparation

Preparation prior to day of assay
Carbonate/bicarbonate buffer – pH 9.6

- Weigh out 1.59g Na₂CO₃ (Proanlys SL 969)
  2.93g NaHCO₃ (Proanlys SL 900)
- Dissolve in 800ml nanopure water
- Adjust pH to 9.6 and make up to a total volume of 1 litre with nanopure water
- Store in a glass bottle at 4°C for 1 week

Phosphate buffered saline (10 x PBS) pH 6.6

1.38 M NaCl (Mwt 58.44) 162g
28mM Na₂HPO₄,12H₂O (Mwt 358.15) 20.04g
11mM NaH₂PO₄,2H₂O (Mwt 156.01) 3.3g
• Weigh out the above chemicals in a 2 litre beaker and dissolve in 1.91 nanopure water
• Adjust pH to 6.6 with 1M HCl
• Make up volume to 2 L with nanopure water

TMB substrate buffer

- 0.5M Citric Acid (anhydrous) Mwt 192.1 1.01g
- 100mM Na₂HPO₄.12H₂O Mwt 358.15 3.58g

• Weigh out the above chemicals in a 100ml beaker
  • Dissolve in 90mls nanopure H₂O
  • Adjust pH to 5.0 and make up to a total volume of 100mls with nanopure water
  • Store at 4°C indefinitely

TMB in DMSO

• Dissolve 0.1g 3’,3’,5’,5’,5’ – tetramethylbenzidine (TMB) in 10 mls Dimethyl sulfoxide (DMSO).
• Store at in fume cupboard at room temperature indefinitely.

**Preparation on Day of Assay**

Wash solution (PBS-Tween 20 – 0.05%)

• Add 200 ml 10x PBS to 1800 ml nanopure water. (to make 1x PBS)
• Add 1ml Tween 20
• Cover with parafilm and invert gently to mix
Blocking buffer – 5% skim milk

- Weigh 1g milk powder (non fat skim milk)
- Dissolve in 100ml PBS/Tween
- Allow 30 ml/plate

Spit Diluent (0.1% skim milk in Wash Solution)

- To prepare 50ml spit diluent, add 1ml blocking buffer to 49ml wash solution.
- Allow 350 ml for 3 plates.

TMB working solution

- Mix 10ml TMB substrate buffer
  100µl TMB in DMSO
  2.25µl H$_2$O$_2$ (added just before use) 30% w/v
- Need 15ml per plate
- Prepare on day of assay
- Use within 30 minutes (immediately once H$_2$O$_2$ added)

Standard/Antibodies

- Standard – Human IgA Sigma T1010 1mg/ml
- α Human IgA / 7S Nordic SK 2917/02
- α Human IgA (α chain) PO-conjugate Sigma
C.3 Method Protocol

C.3.1 Test Procedure

DAY 1

- Make up 10mls of $\alpha$-human IgA/7S at dilution of 1 in 800 with coating buffer.
  
  Pipette 50μL into all wells except blank wells.
  
<table>
<thead>
<tr>
<th>Volume required for 2 plates</th>
<th>Required dilution</th>
<th>Volume of $\alpha$-human IgA/7S</th>
<th>Volume of coating buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mls</td>
<td>1:800</td>
<td>12.5μL</td>
<td>9987.5μL</td>
</tr>
</tbody>
</table>

DAY 2

- Wash all plates 5x in PBS/Tween 20 solution
- Block all wells with 100μL of 5% skim milk powder/PBS/Tween solution
- Incubate for 60 mins at 37°C

- Make up Standard Dilutions as follows:

<table>
<thead>
<tr>
<th>Standard</th>
<th>Final conc. Standard (μg/ml)</th>
<th>Volume standard</th>
<th>Volume of 5% skim milk/PBS/Tween</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>25.0</td>
<td>25μL of 1mg/ml</td>
<td>975μL</td>
</tr>
<tr>
<td>S1</td>
<td>0.25</td>
<td>20μL of S0</td>
<td>1980μL</td>
</tr>
<tr>
<td>S2</td>
<td>0.13</td>
<td>1000 μL of S1</td>
<td>1000μL</td>
</tr>
<tr>
<td>S3</td>
<td>0.06</td>
<td>1000μL of S2</td>
<td>1000μL</td>
</tr>
<tr>
<td>S4</td>
<td>0.03</td>
<td>1000μL of S3</td>
<td>1000μL</td>
</tr>
<tr>
<td>S5</td>
<td>0.015</td>
<td>1000μL of S4</td>
<td>1000μL</td>
</tr>
<tr>
<td>S6</td>
<td>0.008</td>
<td>1000μL of S5</td>
<td>1000μL</td>
</tr>
<tr>
<td>S7</td>
<td>0.004</td>
<td>1000μL of S6</td>
<td>1000μL</td>
</tr>
</tbody>
</table>

- Make up unknown samples as follows:

  They are usually diluted 1:100, 1:1000, 1:10000
• Wash all plates as before 5x

• **Add** 50μL of Sample to sample wells

  50μL of Standard to appropriate to standard wells

  50 μL of positive and negative Control to appropriate wells

  50 μL of skim milk powder to blank wells

  (Negative control diluted 1:100, Positive control diluted 1:400)

  After loading, plates are incubated at 37°C for 60 mins

  Wash plates 5x as before

  Add 50 μL conjugate to all wells

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Volume for:</th>
<th>Volume of IgA PO conjugate</th>
<th>Volume of 1% milk/PBS/Tween</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1600</td>
<td>10mls – 2 plates</td>
<td>6.3 μL</td>
<td>10mls</td>
</tr>
<tr>
<td>1:1600</td>
<td>20mls – 4 plates</td>
<td>12.5 μL</td>
<td>20mls</td>
</tr>
</tbody>
</table>

  Incubate plates for 60 mins at 37°C.

  Wash plates 5X as before

  Add 50 μL of TMB working solution to all wells

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume required</th>
<th>Volume required</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMB substrate</td>
<td>10mls-2 plates</td>
<td>20mls – 4 plates</td>
</tr>
<tr>
<td>TMB in DMSO</td>
<td>100 μL</td>
<td>200 μL</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>1.5 μL</td>
<td>3.0 μL</td>
</tr>
</tbody>
</table>

  Stop reaction after 10 mins by adding 50 μL of 0.5M H₂SO₄ to all wells.

  Read plates on plate reader at 450nm
C.3.2 Calculations and Computations.

The computer software will calculate the values of the unknown by constructing a standard curve using the 4 parameter equation

\[ Y = \frac{(A-D)}{1+(x/C)^B}+D \]

The standard curve is acceptable when:

- The correlation coefficient is higher than 0.980 for IgA
- The calculated values for standards in the linear portion of the curve are in good agreement with the real values. One outlying value can be omitted from triplicates if the other two values are in good agreement.
- Values calculated for controls are in agreement with the range set by previous analyses or with the stated value in the case where there is a known value.

Unknowns are acceptable when:

- The optical density (OD) falls in the portion of the graph that is linear. If the OD is higher than the standard which is at the highest spot of the linear segment, the specimen should be diluted by a further factor of 2 and re-run. If the OD falls below the standard at the lowest point of the linear section of the standard curve, the results should be reported as being a “NIL DETECTED” result or recorded as being < (STD value) if it is considered to be a very small but measurable amount or return at a lower dilution to confirm “NIL DETECTED”.
- The triplicates are in good agreement with one another. One triplicate can be omitted and the result calculated from the remaining duplicates if that triplicate is clearly an outlying result.
Appendix D

Protocol for the analysis of serum for Neopterin by enzyme linked immunosorbent assay.

D.1 Specimen Details

D.1.1 Specimen Collection

Serum should be collected by venepuncture in the standard way.

Neopterin is sensitive to light. Patient samples should therefore be protected from light by wrapping the collection tube in foil.

The sample is immediately centrifuged to separate out the serum and frozen at –20°C.

D.1.2 Specimen Stability

1.2.1 Neopterin is stable at 4-8°C for 3 days, and at –20°C for six months.

1.2.2 Repeated thawing and refreezing should be avoided.

D.1.3 Specimen Preparation

1.3.1 All samples should be free from microbial contamination. Before storage at –20°C serum or plasma should be separated from blood clots.

1.3.2 Insoluble material should be removed from the samples by centrifugation prior to the test.
D.2. Method

D.2.1 Principle of the Assay

The ELItest Neopterin assay is a competitive enzyme immunoassay using coated microtitre plates. The wells of the microtitre plate are coated with anti-neopterin antibodies (sheep, polyclonal). After addition of the enzyme conjugate (neopterin/alkaline phosphatase conjugate) to standard and control sera and to patient samples the neopterin of the patient samples competes with the neopterin/enzyme conjugate for the binding sites of the coating antibodies to form an immune complex bound to the solid phase (anti-neopterin antibody/neopterin or anti-neopterin antibody/neopterin enzyme conjugate complexes).

Subsequent intensive washing ensures the complete removal of all unbound components. The addition of 4-nitrophenyl phosphate as a substrate solution starts the enzyme reaction in which the alkaline phosphatase contained in the neopterin/enzyme conjugate catalyses the cleavage of the phosphate of 4-nitrophenyl phosphate, thus forming the yellow 4-nitrophenol. The enzymatic reaction is stopped by alkalinisation with sodium hydroxide. The intensity of the colour (measured in optical density OD) depends on the quantity of enzyme bound for a constant reaction time and consequently is inversely proportional to the neopterin concentration in the patient sample. Thus high neopterin values correspond to a low optical density.

The optical density is measured by means of a microtitre plate reader at an absorption maximum of 405nm. The results are calculated by plotting a standard curve (optical density versus concentrations of neopterin standard), from which the neopterin concentrations in the patient samples can be read off directly.
D.2.2 Materials and Reagents

ELItest Neopterin Brahms Diagnostica

1 plate, coated with anti-neopterin antibodies (polyclonal, sheep)

1 plate, uncoated

1 vial, enzyme conjugate (neopterin/alkaline phosphatase conjugate), lyophilized.

1 vial (16ml) enzyme conjugate buffer

2 tablets 4-nitorphenyl phosphate

1 vial substrate buffer

1 vial 2N sodium hydroxide (stop solution)

Washing solution concentrate

Neopterin standards, human serum (6 vials)

Control sera (2 vials)

D.2.3 Equipment

1 black cover sheet

Finn pipetting system – 1-10ml, 40-200µl, 200-1000µl

Multichannel pipettes – 50µl, 100µl

1 litre container for washing solution

Molecular Devices Thermomax Microplate Reader

IBM Personal Computer

Printer

Power Supply

Absorbent wash cloths

Pipette tips

Measuring cylinders – 500ml, 20ml, 10ml
D.2.4 Stability and Storage of kit

Store all reagents and the coated microtitre plate (A) at 4-8°C in their original shipping containers until immediately prior to use. Uncoated microtitre plates (A2) can be stored at room temperature.

Adjust the kit components to room temperature before use (about 30 minutes beforehand) and store cool again after use. If a fraction of the standard, the controls, the enzyme conjugate buffer, the substrate buffer, the concentrated washing solution and the stop solution is removed aseptically, the unused quantity of the reagents is stable at 4-8°C in the original containers until the expiry date indicated.

D.2.5 Reagent preparation on day of Assay

Adjust the reagents to room temperature before undertaking the test and ensure the homogeneity of the solution.

Add buffer (C) to the enzyme conjugate (B) to prepare the ready to use form. Stable for at least 4 weeks when kept at 4°C

Prepare washing solution: dilute 11 ml concentrate with distilled water to yield 550ml. Diluted washing solution may be used for up to 4 weeks if stored at 4-8°C. Contaminated washing solution must not be used. This is the case if the liquid is clouded or the pH value is <6.

Ensure the substrate tablets (D) are completely dissolved in substrate buffer (E). This ready to use substrate solution (D+E) is stable for 3 days at 4°C.
D.3 Method Protocol

D.3.1 Test Procedure

Do not interrupt the individual stages in the procedure. Keep the order and the incubation times constant for all samples. The wells in the plate should always be filled in the same order.

Incubation must be carried out in the dark because of the sensitivity of neopterin to light (black cover sheet); the substrate/enzyme reaction can also be carried out in normal daylight.

A standard series and negative and positive controls must be carried out for each microtitre plate.

It is recommended that a blank be determined, for which a corresponding well should be left free. (pipette 100µl of the substrate and add 100µl of the stop solution coincident with adding substrate and stop solution to the rest of the plate).

Duplicates are recommended.

Pipette 50µl of the standards (in increasing concentrations), 50µl of the controls and 50µl of the serum samples each with 150µl of the enzyme conjugate into the uncoated microtitre plate (A2).

Mix thoroughly by shaking briefly or aspirating and ejecting twice.

Transfer an aliquot of 150µl of this mixture into the neopterin-antibody coated microtitre plate (A) using a multichannel pipette. The transfer time should not exceed 5 minutes.

Incubate the microtitre plate for 2 hours at room temperature in the dark (using the black cover sheet).

Aspirate or decant the incubation volume.
Wash all the wells with 350µl of washing solution W. Aspirate or decant the wells again completely. Repeat the last two steps 3 times to ensure a total of 4 washing steps. After the last washing step, remove any remaining droplets by tapping the plate sharply on blotting paper, taking care to ensure no droplets remain in the wells. Pipette 100µl of the dissolved substrate (4-nitrophenyl phosphate) into all wells – including that of the blank. Incubate the microtitre plate (A) again for 30± 5 minutes at room temperature. This reaction can be performed in normal daylight. Because of the temperature dependency of the enzyme activity, it is recommended the incubation of the enzyme/substrate reaction should be as follows:

<table>
<thead>
<tr>
<th>Room Temperature range (°C)</th>
<th>Duration of enzyme/substrate interaction (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17.0-20.4</td>
<td>35</td>
</tr>
<tr>
<td>20.5-24.9</td>
<td>30</td>
</tr>
<tr>
<td>&gt;25.0</td>
<td>25</td>
</tr>
</tbody>
</table>

Pipette 100µl of stop solution F into all wells to stop the enzyme reaction and the formation of colour. Sodium hydroxide must be added in the same order as for the substrate. Ensure the contents of the wells are thoroughly mixed by shaking the microtitre plate (A) briefly before measuring the optical density. The optical density (OD) is measured in a photometer at a wavelength of 405nm. Optionally the measured blank is subtracted from the optical density results obtained.
D.3.2 Calculations and Computations

All commercially available photometer-computer combinations allowing an analysis by spline function or point to point are suitable for the computer-assisted evaluation of the ELItest neopterin.

Alternatively, the results can also be calculated using semilogarithmic graph paper. In this case the standards (x-axis, logarithmic) are plotted against the mean values of the optical density of the standards (y-axis, linear), thus creating a standard curve with the 6 points obtained. The optical density of the samples can now be used to read off the corresponding neopterin concentrations directly in nmol/l.
## Appendix E

Table E.1 Intraclass correlation between variables measured in the PATH study and the same variables measured over a year later in the 2001 study.

<table>
<thead>
<tr>
<th></th>
<th>Intraclass correlation coefficient (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-12 mental health</td>
<td>0.45 (0.35-0.54)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anxiety (Goldberg)</td>
<td>0.52 (0.44-0.60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Depression (Goldberg)</td>
<td>0.47 (0.38-0.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mastery</td>
<td>0.63 (0.56-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ruminative style</td>
<td>0.62 (0.55-0.69)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.67 (0.61-0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure</td>
<td>0.58 (0.50-0.65)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive interaction with friends</td>
<td>0.54 (0.45-0.62)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative interaction with friends</td>
<td>0.59 (0.51-0.66)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive interaction with family</td>
<td>0.48 (0.39-0.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative interaction with family</td>
<td>0.48 (0.39-0.57)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive interaction with partner</td>
<td>0.63 (0.55-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative interaction with partner</td>
<td>0.68 (0.61-0.75)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Negative affect</td>
<td>0.47 (0.38-0.56)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive affect</td>
<td>0.63 (0.56-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hostility</td>
<td>0.33 (0.23-0.44)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Drive</td>
<td>0.72 (0.67-0.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fun-seeking</td>
<td>0.70 (0.64-0.76)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reward responsiveness</td>
<td>0.63 (0.56-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Behavioural inhibition</td>
<td>0.74 (0.69-0.79)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neuroticism</td>
<td>0.77 (0.73-0.82)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Extroversion</td>
<td>0.83 (0.80-0.87)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Psychoticism</td>
<td>0.66 (0.60-0.73)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Marital status</td>
<td>0.95 (0.94-0.96)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Employment status</td>
<td>0.87 (0.84-0.90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Self rated health</td>
<td>0.55 (0.47-0.63)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Decision authority</td>
<td>0.76 (0.71-0.81)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Skill discretion</td>
<td>0.18 (0.07-0.30)</td>
<td>0.001</td>
</tr>
<tr>
<td>Job demands</td>
<td>0.63 (0.56-0.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SF 12 physical health</td>
<td>0.35 (0.25-0.45)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Appendix F

Calculation of equivalent income.

Data collected included gross weekly personal and household income, spouse’s occupation, number of children, ages of children and whether they lived at home part-time, full-time or not at all. Personal income was set at the mean of the range of income indicated for both personal and household income. Spouse gross income was calculated by subtracting personal income from household income. The resulting figure was checked against spouse’s occupation and marital status, i.e. if personal income was identical to household income, checks were made that there was either no spouse, or that the spouse was not working. Annual gross personal and spouse income were calculated (by multiplying weekly income by 52).

Disposable personal and spouse income were calculated by reference to Tax Pack 2001 [602]. Only personal income tax was taken into account in calculating approximate disposable income. Disposable household income was then calculated as the sum of the personal and spouse disposable income.

Points were assigned to a household depending on household characteristics as outlined for the simplified Henderson equivalence scale (as modified by NATSEM [603]. This scale uses the labour force status of parents as well as age and dependency status of children to calculate points. A dependent child was defined according to the definition used by the Australian Bureau of Statistics [604] as:

- All persons less than 15 years of age
- Persons aged 15-24 years who are full-time students, live with a parent, guardian or other relative and do not have a spouse or offspring of their own living with them
There were no available data on the labour force status of children; so that persons aged 15-24 were considered to be dependent if they lived with parents more than half time. In those situations where parents were living in separate accommodation and children spent time in both places, children accrued half of the points of a fully dependent child (clearly this misses the determination of which parent has financial responsibility for non-custodial children).

Table F.1 “Individual points’ for the simplified Henderson equivalence scale (as modified by NATSEM)

| Reference person, working | 20.00 |
| Reference person, not in the labour force or part time | 13.00 |
| Partner, working | 18.50 |
| Partner, not in the labour force or part time | 9.50 |
| Dependent child, 0-5 years | 5.080 |
| Dependent child, 6-14 years | 8.355 |
| Dependent child, 15-24 years | 12.025 |

Table F.2 “Household points” for the simplified Henderson equivalence scale

<table>
<thead>
<tr>
<th>Household size</th>
<th>Housing</th>
<th>Fuel/power</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.1</td>
<td>4.9</td>
<td>17.0</td>
</tr>
<tr>
<td>2</td>
<td>13.3</td>
<td>6.7</td>
<td>20.0</td>
</tr>
<tr>
<td>3</td>
<td>14.5</td>
<td>8.0</td>
<td>22.5</td>
</tr>
<tr>
<td>4</td>
<td>15.7</td>
<td>9.3</td>
<td>25.0</td>
</tr>
<tr>
<td>5</td>
<td>16.9</td>
<td>10.6</td>
<td>27.5</td>
</tr>
<tr>
<td>6</td>
<td>18.2</td>
<td>11.8</td>
<td>30.0</td>
</tr>
<tr>
<td>7</td>
<td>19.4</td>
<td>12.6</td>
<td>32.0</td>
</tr>
<tr>
<td>8</td>
<td>20.0</td>
<td>14.0</td>
<td>34.0</td>
</tr>
<tr>
<td>9</td>
<td>21.2</td>
<td>14.8</td>
<td>36.0</td>
</tr>
<tr>
<td>10</td>
<td>21.8</td>
<td>16.2</td>
<td>38.0</td>
</tr>
<tr>
<td>11</td>
<td>22.4</td>
<td>17.6</td>
<td>40.0</td>
</tr>
<tr>
<td>12+</td>
<td>24.2</td>
<td>19.8</td>
<td>44.0</td>
</tr>
</tbody>
</table>

The total points for the household (individual and household) are summed. The equivalence ratio was then calculated by comparison to the standard income unit comprising a full time worker, a partner not working full time and two dependent
children (67.94). Disposable household income was then divided by the adjusted points to give the equivalent household income.

\[ i.e. \ S = \frac{P}{P_S} \]

where \( S \) = equivalence ratio

\[ P = \text{the total points for the income unit} \]

\[ P_S = \text{the points for a standard income unit (67.94)} \]

The equivalence ratio is then used to adjust the household disposable income to give an estimate of the equivalent income,

\[ I_e = \frac{I}{S} \]

Where \( I_e \) = the equivalent income (adjusted for household circumstances)

\[ I = \text{disposable household income} \]

\[ S = \text{the equivalence ratio} \]