EFFECTS OF DIETARY CHANGES ON ADIPOSE TISSUE METABOLISM, CARBOHYDRATE TOLERANCE AND INSULIN SECRETION IN MAN

by

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STATEMENT

The work described in this thesis was performed in the Department of Clinical Science, John Curtin School of Medical Research, Australian National University, Canberra. My scholarship was awarded by Chulalongkorn University, Bangkok.

Data for preliminary experiments in Chapter 2 were supplied by Dr. R.B. Goldrick, who also performed adipose tissue biopsies throughout this study. Figures for caloric intake and dietary composition were provided by Mrs. N.L. Quinlivan. All other experiments were done by myself under the supervision of Dr. R.B. Goldrick.

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TABLE OF CONTENTS

STATEMENT ii
ACKNOWLEDGEMENTS iii
ABSTRACT viii
ABBREVIATIONS xiii

CHAPTER 1. INTRODUCTION

PREFACE 1
AIMS OF STUDY 7

1.1 METABOLISM OF HUMAN ADIPOSE TISSUE IN VITRO 10
(a) De novo Synthesis of Fatty Acids 12
(b) Lipoprotein Lipase Activity 16
(c) Effects of Fat Cell Size on Adipose Tissue Metabolism 19

1.2 ACTIONS OF INSULIN ON ADIPOSE TISSUE 22
(a) Glucose Transport System 22
(b) Insulin Receptors 23
(c) Glucose Metabolism 24
(d) Amino Acid Transport and Protein Synthesis 25
(e) Antilipolytic Effects 25
(f) Direct Effects on Synthesis of Fatty Acids and Glycogen 31

1.3 EFFECTS OF INSULIN ON GLUCOSE METABOLISM OF ADIPOSE TISSUE IN VITRO 33
(a) Insulin Stimulation of Glucose Metabolism in Rat Adipose Tissue 33
(b) Effects of Insulin on Glucose Metabolism in Human Adipose Tissue 35
   (i) Effect of insulin on the distribution of labelled glucose in adipose tissue glycerides 38
   (ii) The role of adipose cell size on insulin sensitivity of adipose tissue 38
   (iii) Effects of dietary changes on insulin responsiveness of adipose tissue 39
### 1.4 Hormonal Controls on Lipid Mobilization in Human Adipose Tissue in Vitro

- **(a) Lipolytic Pathway**
  - (i) Influence of glucose concentrations
  - (ii) Adipose cell size and lipolysis
- **(b) Antilipolytic Effects of Insulin**
- **(c) Other Antilipolytic Agents**

### 1.5 Insulin Secretion

### 1.6 Role of the Liver in Regulation of Blood Glucose Homeostasis in Humans

- **(a) Hormonal Controls**
  - (i) Insulin
  - (ii) Glucagon and catecholamines
  - (iii) Growth hormone and glucocorticoids
- **(b) Effects of Dietary Changes on Carbohydrate Tolerance in Relation to Hormonal Regulation**
  - (i) Starvation
  - (ii) Carbohydrate deprivation
  - (iii) Isocaloric high carbohydrate feeding
  - (iv) Overfeeding

### Chapter 2. Materials and Methods

- **2.1 Subjects and Dietary Manipulations**
- **2.2 Adipose Tissue Biopsy**
- **2.3 Preparation of Adipose Tissue Samples**
- **2.4 General Conditions of Incubation**
- **2.5 Determinations of Adipose Cell Size and Number**
- **2.6 Estimation of Lipolysis and Esterification in Adipose Tissue**
- **2.7 Preliminary Study Using the Balance Method of Vaughan**
- **2.8 Measurement of Glycerol Concentration in the Incubation Medium and Plasma**
- **2.9 Measurement of FFA Concentration in Adipose Tissue, the Incubation Medium and Plasma**
- **2.10 Blood Analyses**
- **2.11 Radioactive and Chemical Reagents**
- **2.12 Statistical Analyses**
- **2.13 Discussion on Methodology and Some Aspects of Experimental Design**
CHAPTER 3. EFFECTS OF HYPOCALORIC, EUCALORIC AND
HYPERCALORIC DIETS ON LIPOLYSIS,
ESTERIFICATION AND TISSUE FREE FATTY ACID
CONCENTRATIONS IN HUMAN ADIPOSE TISSUE

3.1 INTRODUCTION 98
3.2 MATERIALS AND METHODS 100
3.3 RESULTS 105
(a) Effects of High Carbohydrate and High Fat Meals 105
(b) Effects of Isocaloric High Carbohydrate Diet 107
(c) Effects of Overfeeding with Glucose 109
(d) Effects of Overfeeding with Saturated Fat 111
(e) Effects of Overfeeding with Polyunsaturated Fat 112
(f) Effects of Semi-starvation in Obese Subjects 114
(g) Effects of Semi-starvation in Non-obese Subjects 116
(h) Tissue FFA Concentrations (T_0, FFA) 118
(i) Inter-relationships between Adipose Cell Weight and
Parameters of Metabolism in Adipose Tissue 120
(j) Relationships between Adipose Cell Size, Basal
Concentrations of Plasma Glycerol, Insulin,
Triglycerides, Cholesterol and FFA 124
3.4 DISCUSSION 126
3.5 SUMMARY 133

CHAPTER 4. EFFECTS OF OVERFEEDING ON CARBOHYDRATE
TOLERANCE, INSULIN SECRETION,
ESTERIFICATION AND LIPOLYSIS IN MAN

4.1 INTRODUCTION 137
4.2 MATERIALS AND METHODS 138
4.3 RESULTS 140
(a) Effects of Supplementing the Eucaloric High
Carbohydrate Diet with Glucose 140
(b) Effects of Supplementing the Standard Eucaloric
Diet with Saturated Fat 142
4.4 DISCUSSION 144
4.5 SUMMARY 146

CHAPTER 5. GENERAL DISCUSSION 148

APPENDIX 1. CALORIC INTAKE AND DIETARY COMPOSITION
(STUDIES OF ADIPOSE TISSUE METABOLISM) 165
APPENDIX 2.  CALORIC INTAKE AND DIETARY COMPOSITION  
(STUDIES OF CARBOHYDRATE TOLERANCE AND  
INSULIN SECRETION)  
170

APPENDIX 3.  TISSUE FFA CONCENTRATIONS AND PARAMETERS  
OF METABOLISM IN ADIPOSE TISSUE IN THE  
EUCALORIC STATE  
172

BIBLIOGRAPHY  
174
ABSTRACT

This study was undertaken to determine the changes in metabolic activities of human adipose tissue in vitro (lipolysis, esterification and other metabolic parameters), in response to the administration of a eucaloric high carbohydrate diet, a hypocaloric diet and a hypercaloric, high carbohydrate or high fat diet. Carbohydrate tolerance and insulin secretion as well as the indirectly estimated rates of lipolysis and esterification in vivo were also examined in subjects overfed with a high carbohydrate or high fat diet. Information obtained from the studies in vivo was used to explain the cause of changes in adipose tissue metabolism observed in vitro during overfeeding regimens.

Lipolysis and esterification in adipose tissue were determined on biopsy samples of subcutaneous fat, by a modification of the balance technique of Vaughan. Glycerol release from the tissue was taken as an index of lipolysis, whereas the esterification rate was calculated from the difference between the rate of lipolysis and net change in free fatty acid (FFA) concentrations (tissue plus medium) in the incubation system. Serial adipose tissue biopsies were performed on subjects, before and during dietary manipulations, 1½ hours after a standard high carbohydrate breakfast, which was fed daily throughout the study period. The latter meal contained 750 calories, providing 8% of total calories from protein, 25% of calories from fat and 67% of calories from carbohydrate (P:F:C = 8:25:67%). The amount and composition of diets taken at
lunch and dinner were adjusted to provide the appropriate daily requirement of calories, protein, fat and carbohydrate. Metabolic variables in adipose tissue (lipolysis, esterification, FFA release, change in tissue FFA, net change in FFA in the assay system and tissue FFA concentration) obtained from subjects (after the standard breakfast) on the eucaloric standard diet (P:F:C = 15:39:46%) were compared with those observed on the various dietary manipulations. In subjects participating in the hypocaloric feeding regimen, adipose tissue samples were taken in the postabsorptive state, both in the control and semi-starvation periods. Blood samples were taken every week-day in the postabsorptive state for plasma glucose, insulin, cholesterol, triglycerides, glycerol and FFA estimations.

A eucaloric high carbohydrate diet (P:F:C = 13:13:74%), given to subjects for 4-6 days, elevated basal plasma triglycerides and lowered basal plasma cholesterol, but had no effect on any metabolic parameters measured in adipose tissue in vitro. Human adipose tissue responded to changes in caloric balance in the following manner. The eucaloric high carbohydrate diet supplemented with an additional 30% of calories from glucose (P:F:C = 11:10:79%), administered to subjects for 4-9 days, led to increases in the rate of esterification and net uptake of FFA by adipose tissue, without affecting the rate of lipolysis. These changes may be an adaptive mechanism occurring in adipose tissue for clearance of plasma triglycerides synthesized, in response to a high carbohydrate content in the diet. Furthermore, the hypercaloric high carbohydrate diet lowered the concentrations of plasma cholesterol as well as FFA, and raised the plasma insulin level. The euclaoric standard diet supplemented with an additional 50% of calories as saturated fat (P:F:C = 11:56:33%, p/s ratio of 0.14), for 8-12 days, elevated plasma
glycerol concentration, but caused no changes in the other plasma constituents. The rate of esterification and net FFA uptake by adipose tissue were unaltered, but the rate of lipolysis was moderately stimulated. Overfeeding with the eucaloric standard diet supplementing with 50% of total calories from polyunsaturated fat (P:F:C = 11:57:32%, p/s ratio of 1.26), for 15 days, resulted in decreases in basal plasma triglycerides and cholesterol concentrations. The basal plasma glucose concentration rose. Changes in the metabolism of adipose tissue were comparable to those induced by overfeeding with saturated fat. The difference responses in adipose tissue metabolism, in the absorptive state, to overfeeding with a high carbohydrate and high fat diet may reflect the different states of insulin secretion and insulin sensitivity in vivo. The basal plasma insulin level probably determined insulin secretory response to the high carbohydrate meal. In subjects taking the hypercaloric high carbohydrate diet, the rise in basal plasma insulin concentration might be a determining factor for the increased rate of esterification in adipose tissue. An impairment of the anti-lipolytic effect of insulin or decreased insulin secretion following the meal (induced by the hypercaloric high fat diet) was expected to be a cause of increased lipolysis in adipose tissue in vitro, since the basal plasma insulin level was unaltered.

In the postabsorptive state, the rate of lipolysis and net FFA production in the incubation system were markedly enhanced by a restricted calorie diet (providing daily deficits of 3,150 calories for 1-3 weeks in obese subjects, or of 1,900-2,400 calories for 8 days in non-obese subjects). The hypocaloric diet caused no change in the rate of esterification in adipose tissue in vitro. The basal concentrations of plasma FFA and glycerol significantly rose. The postabsorptive
concentrations of plasma glucose and insulin tended to fall during semi-starvation. These findings indicate that the mobilization of FFA from adipose tissue during caloric deprivation is primarily regulated by an increased breakdown of triglycerides. The decrease in the plasma insulin level is probably responsible for the mobilization of stored substrate (FFA in adipose tissue triglycerides) during diminution of exogenous energy supply.

Overfeeding with glucose or fat induced a decrease in adipose tissue FFA levels (observed after a 30 minute preincubation of adipose tissue). In obese subjects submitted to the hypocaloric feeding, tissue FFA concentrations were reduced, reflecting the displacement of the elevated preformed FFA in vivo. This finding was not observed in non-obese subjects on the semi-starvation diet.

In order to find whether changes in insulin secretion or insulin sensitivity induced by the hypercaloric high carbohydrate and high fat diets were responsible for the different changes in adipose tissue metabolism (observed in vivo in the absorptive state). The responses of blood glucose, plasma concentrations of insulin, glycerol and FFA to the standard high carbohydrate breakfast were recorded in healthy young adults. The results obtained in subjects taking the eucaloric standard diet were compared with those observed during either overfeeding with glucose or saturated fat. Both of the latter diets contained of similar composition to those used in the studies of adipose tissue metabolism in vitro. Overfeeding with the high carbohydrate diet enhanced carbohydrate tolerance without any changes in the insulin secretory response. This indicates an increased sensitivity to the effects of endogenous insulin. Conversely, there was an impaired insulin sensitivity in subjects overfed with the high fat diet. Carbohydrate tolerance was
decreased, whereas insulin secretion was unaltered. Esterification of FFA in adipose tissue *in vivo* (in the postabsorptive or absorptive state) was stimulated by both hypercaloric diets, as indirectly assessed by measuring the plasma FFA and glycerol concentrations. This metabolic change occurred independently of the composition of the diet. The hypercaloric high carbohydrate diet had no effect on the plasma glycerol concentrations recorded in the basal state or following the standard meal. When subjects were overfed with the high fat diet, the basal plasma glycerol concentration was unaffected. In contrast, the plasma glycerol concentrations measured after ingestion of the standard meal were markedly increased, indicating an impairment of the antilipolytic effects of endogenous insulin on adipose tissue. Therefore, changes in adipose tissue metabolism *in vitro* in the absorptive state (induced by overfeeding with a high carbohydrate or high fat diet) reflect different states of insulin sensitivity.

In subjects on the eucaloric standard diet, the rates of lipolysis and esterification, and most of the other metabolic variable in adipose tissue (recorded in the postabsorptive state) were positively correlated with the fat cell weight. Some of these correlations were found to be influenced by nutritional state of the subjects, since there were fewer significant relationships noted after ingestion of the high carbohydrate breakfast. Partial correlation analysis of metabolic parameters in the postabsorptive and absorptive states showed that FFA release was directly proportional to the rate of lipolysis, and the latter was a direct function of the fat cell weight. There was no relationship between the rate of lipolysis and esterification, and each was a separate function of the fat cell weight.
ABBREVIATIONS

cyclic AMP  cyclic-3',5'-adenosine monophosphate
FFA  free fatty acids
GTT  glucose tolerance test
KRP  Krebs-Ringer phosphate buffer
LPL  lipoprotein lipase
VLDL  very low density lipoproteins

Sections, figures and tables are shown by two numbers. The first number indicates the chapter and the second denotes the number of the section, figure or table within the chapter.
Storage and mobilization of triglycerides are functions of adipose tissue, and are regulated by several factors such as caloric requirements, availability of substrates, hormones, and enzyme activities in adipose tissue. Nutritional factors can modify adipose tissue metabolism. Both carbohydrate and fat serve as major substrates for deposition of body fat in a state of positive caloric balance. Conversely, hydrolysis of triglycerides occurs during a state of negative caloric balance, providing free fatty acids (FFA) for energy metabolism of other tissues. Synthesis of triglycerides from carbohydrate in human adipose tissue is achieved by transformation of glucose carbon to alpha-glycerophosphate which is deposited as glyceride-glycerol moiety. The utilization of glucose by human adipose tissue in vitro for fatty acid synthesis de novo is negligible, whereas rat adipose tissue is a major site for fatty acid synthesis. Adipose tissue is capable of taking up triglyceride-fatty acids in triglycerides, which are carried in very low density lipoproteins (VLDL) and chylomicrons in the plasma, and which are hydrolyzed by lipoprotein lipase (LPL) present in the adipose tissue. The FFA released are taken up and esterified with alpha-glycerophosphate and represent the major source of adipose tissue triglyceride-fatty acids.

In man, administration of a eucaloric high carbohydrate diet is usually
associated with hypertriglyceridaemia. Adipose tissue is one of the
major sites for the removal of triglycerides from the circulation,
mediated by an increased activity of its LPL. The latter phenomenon is
possibly accompanied by an enhancement of FFA uptake of the tissue.
Furthermore, increased incorporation of labelled glucose into
triglycerides in adipose tissue reflects stimulation of the
esterification of fatty acids, which was observed in vitro after taking
the high carbohydrate diet. Thus, the above mechanism occurring in
adipose tissue may be responsible for the clearance of hypertriglyceri-
daemia induced by a high carbohydrate diet. In the past 15 years, human
adipose tissue has been studied in vitro as a model to elucidate its
metabolism and regulatory factors in vivo. However, there is no data to
indicate that an administration of a high carbohydrate diet in man
results in stimulation of the net FFA uptake by adipose tissue, leading
to increased esterification. On the other hand, the latter event
perhaps simply reflects an increased rate of triglyceride turnover in
the adipocytes.

When high fat diets are consumed, dietary fatty acids serve as
substrates to be esterified and subsequently deposited in adipose tissue.
In this situation, the utilization of carbohydrate by adipose tissue is
reduced to only provide an adequate supply of alpha-glycerophosphate for
use in the esterification of FFA. Feeding a high fat diet to rats
brings about the inhibition of de novo fatty acid synthesis and
dimination of insulin effects on fatty acid synthesis as well as on
suppression of lipolysis in adipose tissue in vitro. In humans,
isocaloric substitution of fat for dietary carbohydrate is accompanied
by suppression of: basal and insulin stimulated glucose oxidation,
fatty acid synthesis from pyruvate and triglyceride synthesis from
glucose \textit{in vitro}. These findings indicate that the latter diet decreases the utilization of glucose and the response of adipose tissue to insulin. There is also no available information concerning the effect of a high fat diet on the rate of uptake and esterification of FFA in human adipose tissue. On the other hand, the reports on adipose tissue LPL activity in response to a high fat meal are contradictory.

Overfeeding with carbohydrate or fat results in an increase of adipose tissue mass, which is a consequence of enlargement of fat cells. The mechanism(s) whereby human adipose tissue accumulates its triglyceride content as the cells become enlarged have not been defined. It is interesting to know how hypercaloric diets modify the metabolic events in human adipose tissue during deposition of an excessive amount of carbohydrate or fat. The effects of dietary changes on several parameters of metabolism in adipose tissue after induction of experimental obesity in man have been studied. However, only one study has been performed while the subjects gained weight. This study has shown that the hypercaloric high carbohydrate diet causes increased incorporation of glucose into adipose tissue triglycerides and increased capacity of enzymes for de novo fatty acid synthesis, in the presence of insulin \textit{in vitro}.

In obesity, there is an increased rate of triglyceride turnover in the adipocytes. In a state of caloric restriction, obese subjects exhibit an elevated rate of lipolysis in adipose tissue \textit{in vitro}. Changes in the uptake of FFA and esterification during caloric deprivation in adipose tissue have been controversial; they were inhibited in some studies or were unchanged in one study. In the fasted rat, fatty acid synthesis and esterification are inhibited. Thus the same findings probably occur in starved man. On the other hand, if
there is a relationship between rate of lipolysis and esterification in the adipocytes, one can expect an increased esterification if lipolysis is elevated. It is also possible that the rate of esterification may be controlled by hormonal factor(s) \textit{in vivo} during the fasting period.

Lean subjects submitted to fasting also had increased lipolysis. It remains to be seen whether any other metabolic changes which occur in response to starvation in normal weight persons are similar to those observed in obesity.

It is well established that enlarged human adipose cells have increased enzyme activities, indicating greater metabolic activity than their smaller counterparts. Both basal lipolysis and esterification are functions of fat cell size, as observed in man and animals. In enlarged rat fat cells, elevated lipolysis leads to an accumulation of tissue FFA, which in turn potentiates esterification. At present, there is no information confirming such a sequence of events in enlarged human fat cells.

Adipose tissue is one of the major sites for insulin action. The utilization of glucose by human adipose tissue \textit{in vivo} and \textit{in vitro} is augmented by physiologic amounts of insulin. Lipid mobilization is in part regulated by the antilipolytic effect of insulin. In fact, only a small quantity of intravenous or oral glucose load is taken up by adipose tissue. In humans, the liver is an important site for glucose disposal, which is primarily determined by insulin. The amount of calories consumed and the dietary composition influence the carbohydrate tolerance. An improvement of carbohydrate tolerance without alteration of insulin secretory response was observed in normal subjects fed isocalorically with a carbohydrate enriched diet. This finding indicates an increased sensitivity of liver and other tissues to
endogenous insulin. Conversely, a caloric restricted or a eucaloric low carbohydrate-high fat diet causes an impairment of glucose tolerance with a decreased release or a delayed hyperresponse of insulin secretion. The latter observations show the decreased insulin effects on carbohydrate utilization.

Nutritional status and diet composition of the adipose tissue donors also modify the effect of insulin on carbohydrate metabolism of adipose tissue in vitro. Eucaloric and hypercaloric high carbohydrate diets enhance the action of insulin on glucose metabolism in adipose tissue in vitro; whereas a caloric restricted diet or a eucaloric diet containing low carbohydrate-high fat content has the opposite effect. Therefore, any metabolic changes observed in adipose tissue in vitro possibly reflect different states of insulin secretion or insulin sensitivity induced by dietary status of the donors before sampling of adipose tissue.

Increased adipose tissue mass is usually associated with hyperinsulinaemia and insulin resistance. Enlarged human fat cells are sensitive to the effects of insulin in vitro as are smaller cells, only an impaired insulin effect on the oxidation of glucose has been observed in large adipocytes. The latter observation in vitro indicates a residuum of impaired insulin sensitivity in vivo, which has been demonstrated in the liver and muscle. The cause(s) of insulin resistance associating with obesity are disputed. Overfeeding with a basic diet or a high carbohydrate diet has been shown to increase insulin secretion without change in carbohydrate tolerance or insulin sensitivity. There is no information on the effects of overfeeding with a high fat diet on glucose disposal or insulin secretion and sensitivity, or lipolysis and esterification in man. A eucaloric high fat diet has no effect on the
basal lipolysis in human adipose tissue *in vitro*, but appears to reduce the effect of insulin on glucose metabolism in human adipose tissue. The latter event reflects an adaptive mechanism of the body during deposition of dietary fatty acids into adipose tissue triglycerides. If this phenomenon constantly occurs during overfeeding with a high fat diet, it may indicate that a state of insulin resistance developed during increasing body fat mass induced by this type of feeding.
AIMS OF STUDY

The aims of this study are the following:

(1) To determine to what extent the rate of lipolysis and esterification in adipose tissue control the deposition and mobilization of fat in man.

(2) To elucidate whether the rate of FFA uptake by adipose tissue is modified by nutritional status and dietary composition.

(3) To determine if changes in adipose tissue metabolism in vitro (induced by dietary manipulations) observed in the absorptive state are representative of metabolism existing in vivo in the postabsorptive state.

(4) To find out whether lipolysis, esterification and other parameters of metabolism in adipose tissue in the eucaloric state are related to adipose cell size, and to what extent any such relationship can be perturbed by changes in nutritional status.

(5) To evaluate the effects of overfeeding on carbohydrate tolerance and insulin secretion and determine whether these parameters are related to changes in lipolysis and esterification in adipose tissue as assessed indirectly by measuring plasma glycerol and FFA concentrations.

The above aims have been studied by the following experimental designs:

(1) Lipolysis and esterification in human adipose tissue in vitro were measured simultaneously by a modified balance method of Vaughan.
The net change in FFA in the incubation system was also obtained. The contributions of lipolysis and esterification in the control of fat deposition and mobilization in adipose tissue were assessed in individual subjects during one or two weeks of feeding a eucaloric high carbohydrate diet, or hypercaloric high carbohydrate and high fat diets, or a hypocaloric diet. Serial adipose tissue biopsies were performed 2 or 3 times per week, 1½ hours after a standard carbohydrate breakfast. The breakfast was fed daily to all subjects throughout the study periods. Only in subjects participating in the caloric restriction regime, were fat biopsies performed after an overnight fast. By comparing data recorded in the control period (taking a eucaloric standard diet) and in the experimental period, the changes in esterification, lipolysis and net FFA uptake and release during the different dietary manipulations were estimated.

(2) Serial blood samples were taken in the postabsorptive state for determinations of plasma glucose, insulin, FFA, glycerol, triglyceride and cholesterol concentrations, before and during dietary manipulation.

(3) Two series of inter-relationships between adipose cell weight and parameters of metabolism in adipose tissue in the eucaloric state (lipolysis, esterification, tissue FFA concentration, FFA release and change in tissue FFA concentration) were analysed. One series was recorded in the postabsorptive state, the other series was obtained in the absorptive state (1½ hours after the standard breakfast).

The object of studying adipose tissue metabolism in vitro after the standard breakfast was to determine the situation existing in adipose tissue whilst a particular diet was being absorbed. The data obtained are assumed to be representative of the overall effects of dietary
changes (composition or caloric intake) at lunch and dinner. These data were then compared to the changes in concentrations of metabolites in blood in the postabsorptive state. However, precise reasons for the changes observed in the *in vitro* studies could not be given unless the responses of metabolites in blood following the standard breakfast were examined.

(4) Subjects were maintained on a standard eucaloric diet and then were placed on either a hypercaloric high carbohydrate or a high fat diet. The concentrations of plasma glucose, insulin, FFA and glycerol in responses to the standard high carbohydrate breakfast were examined. Blood samples were taken before and at half-hourly intervals following the meal for 2 hours in both control and test periods.
1.1 METABOLISM OF HUMAN ADIPOSE TISSUE IN VITRO

One of the main functions of adipose tissue is to synthesize triglycerides, an important reserve of metabolic energy in the body. Adipose tissue is responsive to the states of energy requirement (post-absorptive state, prolonged fasting, or heavy muscular work) by breakdown of triglycerides to provide FFA. Plasma FFA are utilized by various tissues, especially muscles, in the oxidative processes supplying energy metabolism. In vivo, the accumulation and breakdown of neutral lipids are separately regulated by several factors including hormones, compositions of diet and caloric balance. Adipose tissue is still viable in vitro under suitable incubation conditions and its metabolic activity observed reflects in part the metabolic events which occur in vivo. Thus, results obtained from the studies with adipose tissue in vitro probably are informative in evaluating its important physiological functions as well as the abnormal changes relating pathological conditions in the body.

Under the basal conditions in vitro, human adipose tissue has the ability to take up glucose from the incubation medium [Pozza et al., 1963; Björntorp, 1966; Englhardt et al., 1971a]. Furthermore, it is capable of utilizing glucose for the synthesis of total lipids, of which 85% of labelled glucose carbon is recovered as glyceride-glycerol and the rest as glyceride-fatty acids [Hirsch and Goldrick, 1964; Fessler and Beck, 1965; Gries and Steinke, 1967; Goldrick et al., 1969; Smith, 1971]. The per cent distribution of $^{14}$C labelling of lipid classes
obtained from glucose-U-14C is 74% in triglycerides, 23% in diglycerides and traces in the monoglyceride plus phospholipid and tissue FFA fractions [Hirsch and Goldrick, 1964]. Radioactivity from labelled glucose or acetate recovered in glyceride-fatty acids of adipose tissue is present predominantly in palmitic acid [Hirsch and Goldrick, 1964]. Glucose oxidation is one of several functions of adipose tissue [Fessler and Beck, 1965; Goldrick, 1967a; Bray, 1969; Davidson, 1972] as well as glycogen synthesis from glucose [Davidson, 1972; Davidson, 1975]. Acetate-1-14C, incubated with human adipose tissue in the presence of glucose was found in triglycerides, exclusively in glyceride-fatty acid moiety [Gellhorn and Marks, 1961; Hirsch and Goldrick, 1964; Fessler et al., 1967], and in CO2 [Gellhorn and Marks, 1961]. Moreover, the incorporation of pyruvate-3-14C into CO2, glyceride-glycerol and glyceride-fatty acids was observed with adipose tissue incubated in glucose-containing medium [Bray, 1969; Bray, 1972]. Additionally, the tissue can assimilate radioactive palmitic acid and can esterify it by alpha-glycerophosphate (supplied from glucose) to form triglyceride [Hamosh et al., 1963; Hirsch and Goldrick, 1964; Galton, 1968; Arner and Östman, 1974]. As well, the newly formed FFA were released into the incubation medium containing albumin as a fatty acid acceptor [Hamosh et al., 1963; Hirsch and Goldrick, 1964; Lisch et al., 1973].

Rat adipose tissue can synthesize alpha-glycerophosphate, mainly from glucose [Margolis and Vaughan, 1962]; this is used for the esterification of fatty acyl-CoA to form triglyceride [Tzur et al., 1964]. In rats, triglyceride formation is achieved through phosphatidic acid. The latter, derived (in molar terms) from 1 mole of alpha-glycerophosphate and 2 moles of fatty Acyl-CoA, is converted to 1 mole of diglyceride by dephosphorylation. Diglyceride is then combined with
another mole of fatty acyl-CoA to form triglyceride [Steinberg et al., 1961]. Triglyceride synthesis in human adipose tissue was shown to be mediated by this pathway [Galton, 1968]. However, a more recent study has provided evidence also of a monoglyceride pathway for the esterification of fatty acids, since labelled palmitic acid or glycerol (incubated with human adipose tissue in the presence of glucose) is recovered in tissue mono- and diglycerides in significant quantities [Arner and Östman, 1974; Arner et al., 1976].

(a) De novo Synthesis of Fatty Acids

Human adipose tissue incubated under basal conditions, in some reports, showed a minimal or absent incorporation of glucose into glyceride-fatty acids [Fessler and Beck, 1965; Galton, 1968; Owen et al., 1967; Davidson, 1972; Salans et al., 1974]. In these studies, specimens of adipose tissue were obtained during elective surgery or by biopsy (minor surgery or needle aspiration) after an overnight fast from donors who were previously on a weight-maintaining diet. Adipose tissue taken after glucose 80 g given intravenously for 2 hours [Fessler et al., 1967], or 60-90 minutes after a high carbohydrate breakfast [Mellati et al., 1970], has an increased transformation of glucose-1-14C into glyceride-fatty acids. A hypercaloric diet administered to obese subjects for 2-3 weeks resulted in stimulation of fatty acid synthesis from glucose, acetate or pyruvate in adipose tissue [Bray, 1969]. Conversely, caloric deprivation in obese subjects gave reduced utilization of the above substrates for the synthesis of fatty acids [Goldrick and Hirsch, 1964; Bray, 1969]. Composition of the diet consumed prior to adipose tissue sampling also influences fatty acid synthesis in vitro. Sjöström [1973a] reported that fatty acid synthesis
from glucose-U-14C increased 6 to 11 times, as recorded in adipose tissue shreds from obese subjects given a high carbohydrate diet, with moderately hypocaloric balance, for 22 days. Moreover, with positive caloric balance, fatty acid synthesis \textit{in vitro} is a consistent phenomenon, the conversion of acetate and pyruvate are readily observed; the utilization of glucose and citrate for fatty acid synthesis are less but significant [Bray, 1972]. Therefore, the rate of fatty acid synthesis in human adipose tissue \textit{in vitro} is nutritionally regulated.

On the other hand, the proposal that fatty acid \textit{de novo} synthesis takes place in human adipose tissue is disputable. The controversy arises from the observation of Shrago and associates [Shrago \textit{et al.}, 1967; Shrago \textit{et al.}, 1969; Shrago \textit{et al.}, 1971] that the activity of \textit{citrate cleavage enzyme} (a key enzyme in the pathway of fatty acid synthesis) was virtually negligible. Therefore a lack of \textit{de novo} lipogenesis in human adipose tissue was suggested [Shrago \textit{et al.}, 1971]. In considering this conflicting observation, the well-documented pathway of \textit{de novo} synthesis of fatty acids in the cytosol of animal fat cells should be briefly mentioned. Citrate leaves the mitochondria to be present in the cytosol and is cleaved into oxaloacetate and acetyl CoA by the \textit{citrate cleavage enzyme} (ATP-citrate oxaloacetate lyase) [Kornacker and Ball, 1965]. The carboxylation of acetyl CoA to form malonyl CoA is catalyzed by \textit{acetyl-CoA carboxylase} in the presence of ATP and Mn^{++}. Seven moles of malonyl CoA then condense with 1 mole of acetyl CoA to form 1 mole of palmitic acid by the action of \textit{fatty acid synthetase} in the presence of NADPH [Martin and Vagelos, 1965]. Acetate-1-14C, as a precursor, is directly converted to acetyl CoA in the cytosol and enters the metabolic sequence of fatty acid synthesis [Hanson and Ballard, 1967] without the induction of the \textit{citrate cleavage enzyme}. 
Later studies in human adipose tissue (using a sensitive and optimal assay for measuring these enzyme activities) have indicated the presence of sufficient activities of citrate cleavage enzyme, acetyl-CoA carboxylase and fatty acid synthetase in particle free homogenates of tissue derived from obese subjects 1-2 hours after breakfast [Goldrick and Galton, 1974]. This observation is in accord with the reports of Sjöström [1973a], who showed that the conversion of citrate (-1-14C or -5-14C) and acetate-1-14C into fatty acids in an optimal cytoplasmic assay system were similar. Adipose tissue studied were removed from obese subjects under a slightly hypocaloric high carbohydrate diet regimen [Sjöström, 1973a]. Furthermore, in a study using acetate-1-14C incubated with adipose tissue in the presence of insulin, 14C in palmitic acid distributes in a fashion that supports fatty acid synthesis de novo in the cytosol, not chain elongation in mitochondria [Goldrick and Galton, 1974].

The findings in vitro presumably indicate that human adipose tissue has a capacity for de novo synthesis of fatty acids. The utilization of substrates for lipogenesis is stimulated by positive caloric balance and high carbohydrate content in the diet, and is reduced by negative caloric balance. The activity of enzymes involved in de novo lipogenesis is potentiated by optimal assay conditions.

However, glucose (a physiological substrate) is a poor substrate for fatty acid synthesis, despite stimulation by insulin, as observed in vitro [Bray, 1972; Patel et al., 1975]. Thus, the quantitative importance of this phenomenon in human adipose tissue in vivo is still doubtful. Acetate or pyruvate are suggested to be the more important substrate [Shrago et al., 1969; Goldrick and Galton, 1974], since their actual incorporated rate into fatty acids in adipose tissue in vitro
approached the rate of palmitate production predicted from enzyme activity in homogenates [Goldrick and Galton, 1974]. The physiological significance of acetate or pyruvate lipogenesis in vivo needs further investigation.

Human adipose tissue has a low capacity to utilize glucose, as seen in studies both in vitro and in vivo. The uptake of glucose by adipose tissue in vitro is very small (Östman, 1965; Englardt et al., 1971a); slices of tissue incubated with glucose of 5.6 mM/l assimilate only 0.82 μmole of glucose/g wet weight/hour [Englardt et al., 1971a]. Similarly, 75 minutes after an intravenous injection of 25 g glucose together with 50 μCi of glucose-U-14C to subjects, only 0.4% and 2.0% of injected labelled glucose were found in adipose tissue triglycerides of normal and obese subjects respectively [Björntorp et al., 1971a]. Moreover, Mellati et al. [1970], using data of maximal rate of fatty acid synthesis from glucose in vitro, calculated that the subcutaneous adipose tissue mass of normal weight subjects utilized 13 g of glucose for the synthesis of whole body fat acids in 24 hours. Recently, Sjöström [1973b] has computed that total adipose tissue mass from subjects with obesity has maximal enzyme capacity to transform only 7.7 g of glucose per day for de novo synthesis of fatty acids. He suggested that the latter metabolic parameter is of little quantitative significance for fatty acid storage or for consumption of glucose carbon by human adipose tissue [Sjöström, 1973b].

On the other hand, glucose is essential for deposition of triglycerides; the consumption of glucose carbon (alpha-glycerophosphate) for the esterification of fatty acids is a main function of adipose tissue. It is probable that fatty acids taken up from the hydrolysis of plasma triglycerides are an important source for adipose tissue glyceride-fatty acids.
(b) Lipoprotein Lipase Activity

Lipoprotein lipase (LPL) is an indispensable enzyme for the uptake of triglyceride-fatty acids by the extrahepatic tissues. It hydrolyses triglycerides, carried in plasma in VLDL and in chylomicrons at the surface of capillary endothelial cells [Robinson, 1970]. Studies with rat adipose tissue, using electron microscope and cytochemical analysis, have shown that the hydrolysis of labelled chylomicron-triglycerides occurs within the capillary endothelial cells and at subendothelial space near pericytes [Blanchette-Mackie and Scow, 1971; Scow et al., 1972]. FFA released at the site of hydrolysis are assimilated by adipose cells to be stored as triglycerides or further oxidized [Robinson, 1963; Robinson, 1970]. Extracts of animal adipose tissues possess LPL activity [Korn and Quigley, 1955; Angervall, 1960; Rodbell, 1964a]. The enzyme is liberated from adipose tissue into the incubation medium by the addition of heparin [Cherkes and Gordon, 1959]. Insulin, in the presence of glucose, significantly raises the activity of LPL in acetone-ether extracts of adipose tissue from fed rats [Austin and Nestel, 1968] or starved rats [Salaman and Robinson, 1966; Elkeles, 1974]. The state of nutrition of animals also influences the LPL activity in adipose tissue. Diet rich in carbohydrate [Garfinkel et al., 1967], or a high fat meal [Pokrajac and Lossow, 1967], or fasting-refeeding state [Garfinkel et al., 1967; Bezman et al., 1962], enhances the enzyme activity; while fasting produces an opposite effect [Cherkes and Gordon, 1959; Hollenberg, 1959; Garfinkel et al., 1967].

It has been demonstrated in rats that at least 2 forms of LPL exist in adipose tissue [Nilsson-Ehle et al., 1975a]. With gel filtration, 30-50% of total enzyme activity in extracts of intact tissue is recovered in LPLa (a physiological active form) and the rest is in LPLb
(a protein synthesis dependent precursor). In extracts of isolated adipocytes, the enzyme activity is only half of that in the former preparation, and mainly comprises LPLb (80-97% of total activity). LPLa is released from adipocytes and is present in the incubation medium [Nilsson-Ehle et al., 1975a].

Human adipose tissue has LPL activity, as demonstrated in heparin eluates from intact tissue [Nestel and Havel, 1962; Diengott and Kerpel, 1965; Persson et al., 1966; Persson et al., 1972; Elkeles, 1974; Pykälistö et al., 1975a; Persson et al., 1975], or in acetone-ether extracts of tissue [Nilsson-Ehle et al., 1972; Elkeles, 1974; Nilsson-Ehle, 1974; Björntorp et al., 1975; Pykälistö et al., 1975a]. The heparin releasable enzyme activity was suggested to reflect the capacity of physiological removal of plasma triglycerides; LPL activity in the acetone-ether powder represented the total enzyme activity of adipose tissue [Robinson, 1963; Pykälistö et al., 1975a]. However, the activities of enzymes determined in the two different sources are significantly correlated [Persson et al., 1975; Pykälistö et al., 1975a].

In humans, nutritional condition seems to regulate the activity of LPL in adipose tissue in vitro. Normal subjects subjected to an isocaloric sucrose-rich diet for 2 weeks had an increased LPL activity eluted by heparin from adipose tissue [Cahlin et al., 1973]. Moreover, adipose tissue removed from normal subjects after a high carbohydrate meal [Nilsson-Ehle et al., 1972], or after a glucose intake [Nilsson-Ehle et al., 1975b] had the elevated LPL activity, as compared with activity observed following an overnight fast.

The stimulating effects of high carbohydrate content of the diet or meal on LPL activity probably depend on a rise of insulin secretion. Plasma insulin response to glucose or high carbohydrate diet is directly
proportional to the raised enzyme activity [Nilsson-Ehle et al., 1975b; Pykäläistö et al., 1975b]. However, insulin (10 μg/ml) \textit{in vitro} has no stimulation on the enzyme activity in adipose tissue after an overnight fast [Elkeles, 1974]. Recently, Guy-Grand and Bigorie [1975] found no correlation between adipose LPL activity and plasma insulin in the fasting state. They suggested that the positive relationship between plasma insulin and activity of LPL in adipose tissue was observed only in the postprandial state.

The effect of a high fat meal on LPL activity in adipose tissue is contradictory. A high fat meal (250 ml containing mixed fat and carbohydrate) had a stimulating effect on the heparin elutable LPL activity [Diengott and Kerpel, 1965]. However, no change in enzyme activity (in acetone-ether powder of tissue) was observed after an ingestion of corn oil (40 g) [Nilsson-Ehle et al., 1975b]. Differences in dosage and the composition of fat in the meal may be responsible for the conflicting results.

Negative caloric balance is associated with a diminution of LPL activity in adipose tissue, as observed in obese subjects after complete starvation for 8 days [Persson et al., 1970] or after a 55% reduction of total calories for 1 month [Guy-Grand and Bigorie, 1975]. In the latter observation, caloric restricted diet caused no change in size of adipose cell despite a significant reduction in body weight [Guy-Grand and Bigorie, 1975].

Insulin-dependent diabetic patients had a lower activity of heparin releasable LPL in adipose tissue than in non-diabetic subjects [Guy-Grand and Bigorie, 1975; Pykäläistö et al., 1975b], this defect \textit{in vitro} was restored to normal level after an insulin treatment [Pykäläistö et al., 1975b]. Therefore, insulin probably plays an important role in the
regulation of LPL activity in vivo. From evidence available, it is unclear that insulin exerts its direct action on enzyme activity (in synthesis or activation), or the improved metabolism in diabetic subjects induced by insulin leads to a return of LPL activity in adipose tissue.

In summary, LPL activity in human adipose tissue in vitro is changed by nutritional state of donor prior to tissue sampling, probably mediated through insulin effects.

(c) Effects of Fat Cell Size on Adipose Tissue Metabolism

Enlargement of human fat cells is produced mainly by an accumulation of the lipid content [Englhardt et al., 1971b]. To a much lesser extent, there is an increase in the cytoplasmic compartment, resulting in a markedly reduced proportion of the lipid-free space to the total cell volume [Englhardt et al., 1971b]. Moreover, protein content as well as amounts of phospholipids and cholesterol are positively correlated with fat cell size [Björntorp and Sjöström, 1972]. Furthermore, the expanded cytoplasmic mass with adipose cell enlargement is associated with increases in activities of several enzymes such as lactate dehydrogenase, aldolase, hexophosphate isomerase [Englhardt et al., 1971b]; Björntorp and Sjöström, 1972], hexokinase isoenzyme I and II [Bernstein et al., 1975]. These findings, therefore, suggest that enlarged human fat cells are potentially metabolically more active than the cells of smaller size. This phenomenon is confirmed by the observed metabolic parameters in human adipose tissue in vitro. The basal incorporation of labelled glucose into glyceride-glycerol is directly proportional to the size of the fat cells, as recorded in fragments or
isolated cells of adipose tissue removed from different individuals [Smith, 1970a; Goldrick and McLoughlin, 1970; Smith et al., 1973; Salans et al., 1974]. However, studies using fat cells of different sizes from different subjects do not prove that cell size influences metabolism in adipose tissue, since any observed changes may be partly due to the residual effect of internal milieu of the donors. To avoid this problem, Björntorp and Karlsson [1970] graded fat cells of different size in tissue pieces from one subject by using differential flotation rate. They reported a positive correlation between fat cell size and the rate of esterification of FFA by glucose [Björntorp and Karlsson, 1970]. Smith [1971] used cells of different size separated by incubated tissue with collagenase at different length of time and obtained a similar result. These data indicate that the basal rate of glyceride-glycerol synthesis from glucose is a function of fat cell size regardless of internal environment of adipose tissue donor. 


There are also several reports which show that the rate of basal lipolysis (triglyceride breakdown) in vitro increases with fat cell size, as measured in fat cells of different size from different subjects [Smith, 1970b; Jacobsson and Smith, 1972; Knittle and Ginsberg-Fellner, 1972; Gries et al., 1972; Östman et al., 1973; Arner et al., 1976]. Identical results were reported in a study using fat cells of different size from the same subjects, subcutaneous fat cells with larger size had the greater rate of glycerol production than omental
cells with smaller size [Goldrick and McLoughlin, 1970]. In addition, the adipose tissue contents of mono- and diglycerides as well as FFA in large cells are increased with increasing fat cell size [Arner et al., 1976].

Thus, studies in vitro show increases in both lipid accumulation and mobilization, indicating an accelerated turnover rate of triglycerides in enlarged human adipocytes. A similar phenomenon occurs in vivo. Following the intravenous administration of glucose-U-14C, the amount of radioactivity recovered in triglycerides from biopsies of human adipose tissue is proportional to cell size [Björntorp et al., 1971a]. An increased mobilization of lipids is evident in human obesity. Fat cell enlargement is usually observed in obesity [Björntorp and Sjöström, 1971; Stern et al., 1973] which is accompanied by an elevation of plasma FFA [Opie and Walfish, 1963; Kjellberg and Östman, 1971] and glycerol [Björntorp et al., 1969a]. Moreover, increased rates of turnover of plasma FFA [Nestel and Whyte, 1968] and glycerol [Björntorp et al., 1969a; Bortz et al., 1972] have been noted in obese subjects. It can be concluded that the metabolic changes in enlarged fat cells are in part the cause of increased triglyceride turnover observed in obesity.

The effect of adipose cell enlargement on LPL activity in vitro of human tissue appears to be comparable to the other metabolic parameters. Several investigators have shown a positive relationship between adipose cell size and the enzyme activity, expressed as a per cell basis, in both heparin eluates and acetone-ether powder of adipose tissue [Pykäläistö et al., 1975a,b; Guy-Grand and Bigorie, 1975; Björntorp et al., 1975]. Additionally, the uptake of triglyceride emulsion labelled with palmitic acid-1-14C increases in direct proportion to increasing
size of the fat cells [Björntorp et al., 1975]. It seems likely that adipose cell enlargement is associated with acceleration in two metabolic events, the rate of turnover of triglycerides, and the capacity to hydrolyze plasma chylomicrons and VLDL. The latter metabolic event may reflect an increased rate of triglyceride-fatty acid uptake.

1.2 ACTIONS OF INSULIN ON ADIPOSE TISSUE

(a) Glucose Transport System

One of the insulin actions on this metabolically active tissue is to accelerate a carrier-mediated transport system of glucose across the adipocyte plasma membrane [Crofford and Renold, 1965a,b]. This effect results in an increased rate of glucose uptake which in turn enhances intracellular glucose utilization [Renold et al., 1965]. Studies with rat adipose tissue in vitro [Crofford and Renold, 1965a,b], have shown that glucose enters the adipocytes by a stereospecific mechanism, since both rate and quantity of D-glucose transported through the cell membrane are markedly greater than those of L-glucose. There is a competitive inhibition of glucose transport by 3-o-methyl-glucose (a non-metabolizable glucose analogue), as shown by decreased oxidation of glucose to CO₂ in adipose tissue incubated with glucose plus its analogue [Crofford and Renold, 1965b]. The presence of a trans-membrane concentration gradient of glucose indicates a rate-limiting transport system for the control of glucose metabolism within the adipocytes [Renold et al., 1965]. Insulin acts directly on the glucose transport system at the cell membrane level, since it enhances the rate of glucose entry and increases the intracellular concentration of free glucose in adipose tissue [Crofford and Renold, 1965a]. Furthermore, an insulin-like action of phospholipase C (on the glucose transport system and on fatty...
acid synthesis from glucose in isolated rat fat cells) suggests that insulin alters the lipoprotein configuration of the cell membrane [Rodbell, 1966]. The latter phenomenon facilitates the passage of glucose into the cells [Rodbell, 1966]. Additionally, studies with isolated membrane of fat cells have demonstrated the primary stimulating effect of insulin on glucose transport, independent of its effect on intrinsic glucose metabolism of the adipocytes [Rodbell, 1967].

(b) Insulin Receptors

The first step of hormone-cell interaction is the uptake of insulin by fat cells which is a rapid process and being correlated to the conversion of glucose to CO₂ and total lipids [Crofford, 1968]. The existence of insulin receptors on the surface of fat cell membranes has been established by the investigations of Kono [Kono, 1969a,b; Kono, 1970], that peptide bonds of the receptors are rapidly destroyed by a proteolytic enzyme, trypsin, and are restored by further incubation in the absence of this enzyme. The trypsin-treated isolated fat cells show no response to the effect of insulin (1.0 μu/ml) on the oxidation of glucose or the synthesis of total triglycerides, without any impairment in basal glucose metabolism. These data suggest that glucose can normally enter fat cells by the carrier-mediated transport system, and that the latter system is distinct from the interaction between insulin and its receptors which, presumably, is inhibited by the proteolytic enzyme [Kono, 1970]. Moreover, physiological significance and characteristics of these specific insulin binding sites have been studied on the cell membranes of other tissues, viz. liver [Freychet et al., 1971], lymphocytes [Gavin et al., 1973], skeletal muscle [Olefsky et al., 1976], as well as adipose tissue [Olefsky et al., 1974].
Studies with isolated human fat cells have shown the presence of two types of insulin receptors, high and low affinity sites. Only a small number of the receptors is required for the binding of insulin, at physiological ranges, to induce a maximal glucose uptake by adipocytes [Olefsky et al., 1974]. On the other hand, insulin binding to the receptors appears to be the first step of the hormone-cell interaction which in turn stimulates the glucose transport system, the utilization of intracellular glucose [Gliemann et al., 1974; Crofford, 1975] and the reduction of stimulated cyclic-3',5'-adenosine monophosphate (cyclic AMP) levels in adipocytes [Jerums and Galton, 1974]. However, the mechanism by which these receptors control the glucose transport system and other metabolic parameters in adipocytes is still obscure.

(c) Glucose Metabolism

Insulin has been shown to stimulate the transformation of glucose to triglycerides and CO$_2$ in rat adipose tissue pieces [Winegrad and Renold, 1958a] and isolated fat cells [Rodbell, 1964b]. An increased glucose metabolism in the intracellular metabolic pathways by insulin is a consequence of facilitating glucose entry across the plasma membrane [Flatt and Ball, 1964], thereby increasing the availability of glucose to be phosphorylated and further metabolized in the glycolytic pathways [Renold et al., 1965]. Thus, the increases in intermediate metabolites and enzyme activities lead to accelerated synthesis of triglycerides, fatty acids, glycogen and lactate in this tissue [Winegrad and Renold, 1958a; Lynn et al., 1960; Flatt and Ball, 1964; Rodbell, 1964b; Goldrick, 1967a]. (See detailed discussion in section 1.3 "Effects of insulin on glucose metabolism of adipose tissue in vitro").
(d) Amino Acid Transport and Protein Synthesis

Insulin also enhances protein synthesis in adipose tissue, as shown by an increased incorporation of labelled amino acids into labelled protein content of fat cells [Herrera and Renold, 1965; Crofford et al., 1970]. However, it fails to enhance the transport of a non-utilizable amino acid, α-aminoisobutyric acid, by the plasma membrane of isolated fat cells [Clausen and Rodbell, 1969] or by intact adipocytes [Goodmann, 1966; Crofford et al., 1970]. Crofford et al. [1970] have suggested that the stimulating effect of insulin on protein synthesis from amino acids is, perhaps, a mediation of insulin action on some intracellular processes involved in protein synthesis. However, the exact mechanism is not yet known.

(e) Antilipolytic Effects

Insulin can suppress the release of FFA from rat adipose tissue incubated with epinephrine and glucose [Gordon and Cherkes, 1958]. It has been observed that glucose (2.8 mM/L) enhances the incorporation of palmitate-1-14C into tissue triglycerides, reflecting an increased rate of fatty acid esterification [Steinberg et al., 1960], and that alpha glycerophosphate in adipose tissue is mainly synthesized from glucose and its glycolytic intermediates [Margolis and Vaughan, 1962]. Insulin, therefore, by acceleration of glucose transport into the cell, increases the availability of alpha-glycerophosphate to conjugate with FFA. The latter process results in diminution of FFA release from adipose tissue [Jungas and Ball, 1963].

Insulin exerts direct actions on some metabolic parameters in rat adipose tissue, independently of glucose transport, for example, antilipolytic effect, stimulatory effects on fatty acid and glycogen
synthesis. In the absence of glucose, the antilipolytic effect of insulin at physiological ranges (0.01-0.1 μu/ml) on epinephrine-stimulated lipolysis was demonstrated in incubated rat adipose tissue [Jungas and Ball, 1963] and isolated fat cells [Rodbell and Jones, 1966]. Furthermore, insulin inhibits lipolysis in response to submaximal concentrations of other lipolytic agents, norepinephrine, adrenocorticotropin (ACTH), glucagon, cortisol and theophylline [Jungas and Ball, 1963; Mahler et al., 1964; Rodbell and Jones, 1966]. Moreover, Mahler et al. [1964] have shown that insulin (0.1 μu/ml) lowers the elevated lipolysis in adipose tissue obtained from rats fasted for 24 hours or rats treated with insulin antibody to induce an insulin insufficiency. It is clearly shown that insulin at low dosages (0.001-1.0 μu/ml) exhibits an inhibitory effect on lipolysis induced by submaximal concentrations of catecholamines independently of glucose metabolism in fat cells (Fain et al., 1966; Chlouverakis, 1967; Lavis and Williams, 1975). However, the antilipolytic effect of insulin on basal lipolysis without glucose in the incubation medium is unsettled. Insulin at concentrations of 0.01-10.0 μu/ml fails to reduce basal release of glycerol from rat adipose tissue [Mahler et al., 1964]; whereas insulin at 0.5 μu/ml suppresses the basal glycerol production [Chlouverakis, 1967].

It is likely that insulin has a biphasic action on catecholamine-stimulated lipolysis in rat adipose tissue pieces [Mahler et al., 1964; Chlouverakis, 1967] and isolated fat cells [Lavis and Williams, 1975]. Insulin at physiological dosages (0.5 μu/ml) significantly stimulates glycerol release induced by very high concentrations of epinephrine (20 μg/ml, Chlouverakis, 1967). Additionally, a 10-fold rise in insulin concentration (from 1.0 to 10.0 μu/ml) fails to further inhibit
lipolysis in adipose tissue incubated with submaximal dose (0.1 μg/ml) of epinephrine [Chlouverakis, 1967].

It has been proposed that the antilipolytic action of insulin on catecholamine-stimulated lipolysis, in the absence of glucose, is perhaps mediated by a reduction of cyclic AMP level in adipose tissue [Jungas and Ball, 1963]. Studies with adipose tissue have provided evidence that catecholamines stimulate lipolysis by activating adenyl cyclase at the cell membrane [Klainer et al., 1962]. The rise of adenyl cyclase provokes the accumulation of intracellular cyclic AMP [Butcher et al., 1965; Butcher et al., 1968; Manganiello et al., 1971], which in turn activates a protein kinase [Kissebah et al., 1973]. The latter enzyme stimulates a hormone sensitive lipase in adipose tissue by phosphorylation [Huttunen and Steinberg, 1971].

The evidence that insulin lowers the elevated cyclic AMP induced by catecholamines and other lipolytic agents was reported by Butcher and associates [Butcher et al., 1966; Butcher et al., 1968]. Insulin (1.0 μu/ml) rapidly reduced the intracellular cyclic AMP level in fat pads preincubated with a high dose of epinephrine (10 μg/ml) or with a low concentration of epinephrine (1 μg/ml) plus caffeine (an inhibitor of cyclic nucleotide phosphodiesterase) [Butcher et al., 1966], as well as in isolated rat fat cells exposed to ACTH or glucagon in the presence of caffeine [Butcher et al., 1968]. It was concluded that insulin antagonized the stimulated lipolysis in response to those agents, in part, by its cyclic AMP-lowering effect in adipose tissue [Butcher et al., 1966].

On the other hand, there is no correlation between the antilipolytic and the cyclic AMP-lowering effects of insulin in adipose tissue [Butcher et al., 1966; Pain and Rosenberg, 1972; Khoo et al., 1973].
However, under the condition that lipolysis is induced by a low concentration of catecholamine and tissue cyclic AMP is rate-limiting for lipolysis, both actions of insulin are comparable [Sneyd et al., 1968].

The mechanism of action by which insulin lowers cyclic AMP level in adipose tissue is not fully understood. It appears that insulin neither alters concentration of the non-stimulated cyclic AMP in the tissue nor changes basal glycerol release [Butcher et al., 1966; Manganiello et al., 1971]. It is possible that a substantial amount of tissue cyclic AMP under basal conditions is non-functional. Therefore, if only a small quantity of the latter is suppressed by insulin, there will be no reduction in the rate of basal lipolysis [Butcher et al., 1966]. Alternatively, insulin may antagonize the effects of catecholamines at various steps involving lipolysis. The probability that it may reduce the stimulated-cyclic AMP level by preventing the accumulation of cyclic nucleotide, has been studied: the proposal that insulin may inactivate adenyl cyclase, thereby decreasing tissue cyclic AMP, is still controversial. Evidence of inhibitory effects of insulin on adenyl cyclase was reported by Jungas [1966]. Studies with rat pad fat pre-incubated with insulin have shown a 30% decrease in the rate of ATP-\( ^{14} \)C converted to cyclic AMP-\( ^{14} \)C, as measured in adipose tissue homogenates [Jungas, 1966]. Conversely, insulin does not alter the basal or ACTH-stimulated adenyl cyclase activity in isolated plasma membranes of isolated rat fat cells [Rodbell et al., 1968; Fain and Rosenberg, 1972].

The influence of insulin on cyclic AMP-phosphodiesterase activity, an enzyme covertng cyclic AMP to a non-cyclic nucleotide, in adipocytes has been studied. Senft et al. [1968] observed that adipose tissue obtained from rats in insulin deficient states (alloxan diabetic or
starved rats) had a reduced phosphodiesterase activity in tissue homogenates, which was significantly increased after an intravenous injection of insulin or glucose. They suggested that insulin increases phosphodiesterase activity probably mediated by increased enzyme synthesis, since in studies with liver in vitro actinomycin D (an inhibitor of protein synthesis) inhibits the rise in enzyme activity in the response to insulin. Furthermore, homogenates of rat isolated fat cells contain two cyclic nucleotide phosphodiesterases with different $K_m$ values. Insulin has been shown to increase the $V_{max}$ of the low $K_m$ enzyme [Loten, 1973]. However, insulin, in these following conditions: preincubated with adipose tissue pieces, added in the assay system of enzyme determination, or injected to rats, does not enhance the degradation of dibutyryl cyclic AMP by phosphodiesterase in tissue homogenate [Blecher et al., 1968]. Insulin probably exerts its cyclic AMP-lowering effect by some other mechanism, not entirely by its direct action on adenyl cyclase or phosphodiesterase activity.

The alteration of physical configuration of adipocyte plasma membrane (induced by the hormone-cell interaction [Rodbell, 1966]) may change the activity of the membrane adenyl cyclase system, and probably causes a reduction in the level of tissue cyclic AMP [Sutherland and Robinson, 1969; Fain and Rosenberg, 1972]. However, there is no study conducted to elucidate this hypothesis.

On the other hand, the antilipolytic action of insulin in the absence of glucose may be brought about by an antagonizing effect of insulin on the activation of triglyceride lipase, since insulin depresses the activity of the hormone sensitive lipase with no correlation in the changes of intracellular cyclic AMP levels [Khoo et al., 1973]. Thus, a step linking cyclic AMP action and the activation
of triglyceride lipase is probably blocked by insulin. It appears that cyclic AMP dependent protein kinase, an enzyme activating the triglyceride lipase in adipose tissue, comprises a regulatory subunit (inactive holoenzyme) and an active catalytic subunit [Soderling et al., 1973]. Epinephrine incubated with rat adipose tissue produces a small rise in cellular cyclic AMP levels and increases the catalytic subunit of phosphoprotein kinase which in turn stimulates lipolysis [Soderling et al., 1973]. Insulin depresses epinephrine-stimulated concentration of protein kinase by decreasing the active subunit of the enzyme. However, this effect was thought to be the decreased cyclic AMP level induced by insulin [Soderling et al., 1973]. An hypothesis that insulin produces its antilipolytic effect by the mediation of an increased intracellular calcium concentration was proposed by Kissebah and associates [Kissebah et al., 1974a; Hope-Gill et al., 1974]. Since both procaine hydrochloride (an agent inducing an alteration in cellular calcium distribution) and insulin inhibited lipolysis and depressed protein kinase activity in homogenates of rat adipose tissue or isolated fat cells in the presence of epinephrine, without any change in tissue cyclic AMP level [Kissebah et al., 1974a], they suggested that the increased intracellular free calcium inhibited the protein kinase and stimulated phosphatase(s) in the adipocytes. The latter enzyme acts by dephosphorylating the triglyceride lipase [Kissebah et al., 1974b], resulting in an inhibition of lipolysis [Kissebah et al., 1974a]. However, by which mechanism insulin induces changes in distribution of intracellular free calcium remains to be investigated.
(f) Direct Effects on Synthesis of Fatty Acids and Glycogen

Insulin stimulates synthesis of fatty acids and glycogen in adipose tissue by mechanisms other than increased glucose transport across the fat cell membranes, as shown in studies using adipose tissue from fasted-refed rats incubated with insulin (1.0 μU/ml) but without glucose [Jungas and Ball, 1964]. In respect of increased fatty acid synthesis in adipose tissue, insulin stimulates the activity of pyruvate dehydrogenase, an enzyme oxidizing pyruvate to acetyl CoA [Jungas, 1970; Coore et al., 1971]. This enzyme is unaffected by cyclic AMP added to the assay system. It is inactivated by an ATP-dependent kinase, but is activated by a Mg²⁺-dependent phosphatase which dephosphorylated the pyruvate dehydrogenase. Thus, insulin may act by stimulating the phosphatase resulting in an increased proportion of active pyruvate dehydrogenase [Coore et al., 1971].

Insulin depresses glycogenolysis in adipose tissue of fasted-refed rats [Jungas and Ball, 1964], and increases the incorporation of both glucose-U-¹⁴C and fructose-U-¹⁴C into glycogen [Hope-Gill et al., 1974]. Lipolysis and glycogenolysis in adipose tissue seem to be operated by a common regulatory mechanism, since catecholamines also stimulate the activity of phosphorylase [Vaughan, 1960]. In the liver and muscle, catecholamines and glucagon stimulate the breakdown and inhibit the synthesis of glycogen, via the adenyly cyclase-cyclic AMP system and the phosphorylation of glycogen phosphorylase and synthetase [Soderling et al., 1970; Walsh et al., 1971]. The glycogen phosphorylase is activated whereas the latter enzyme is inactivated by phosphorylation. Furthermore, in the muscle an increase in glycogenolysis is associated with increased phosphorylation of glycogen synthetase and phosphorylase by a cyclic AMP-
dependent protein kinase [Soderling et al., 1970]. Diabetic rats had
decreased glycogen synthetase and increased glycogen phosphorylase
activity in the liver, and both enzyme activities were returned to normal
levels by insulin treatment [Bishop and Larner, 1967]. Additionally,
studies with rat liver have shown that a glycogen synthetase phosphatase
dephosphorylates the glycogen synthetase, and that insulin provokes the
former enzyme activity, resulting in an inhibition of glycogenolysis
without any comparable changes in tissue cyclic AMP levels [Miller and
Larner, 1973]. In adipose tissue, insulin stimulates the synthesis of
glycogen by the stimulation of glycogen synthetase and inhibition of
glycogen phosphorylase, as the result of its cyclic AMP-lowering effect
[Jungas, 1966]. However, there is no information whether a sequence of
enzyme reaction observed in the liver and muscle exists in adipose
tissue, or is influenced by insulin.

In summary, in studies with rat adipose tissue, insulin facilitates
 glucose entry across fat cell membranes. As a result of this effect,
the intracellular metabolism of glucose is augmented. Binding of
insulin to specific receptors on cell surface membranes is the primary
determinant of insulin action. Adipose tissue responds to actions of
insulin by increasing the transformation of glucose to CO₂,
triglycerides, fatty acids, and glycogen. Insulin also stimulates the
incorporation of amino acids into the protein in fat cells.

Insulin has been shown to directly inhibit the stimulated lipolysis
in fat cells possibly via its cyclic AMP-lowering effect. The latter
phenomenon may be brought about by several possible effects of insulin,
i.e., inhibition of adenyl cyclase or stimulation of phosphodiesterase
activities. Insulin probably exerts its antilipolytic effect by
lowering the activity of a protein kinase, resulting in inactivation of
triglyceride lipase in fat cells.

Another direct effect of insulin in rat adipose tissue is enhancement of fatty acid synthesis mediated by the stimulation of pyruvate dehydrogenase activity. As a consequence of reduction in tissue cyclic AMP induced by insulin, the synthesis of glycogen in adipose tissue is also increased.

1.3 EFFECTS OF INSULIN ON GLUCOSE METABOLISM OF ADIPOSE TISSUE IN VITRO

(a) Insulin Stimulation of Glucose Metabolism in Rat Adipose Tissue

The effects of insulin on glucose metabolism in rat adipose tissue incubated in vitro have been well documented. Insulin in vitro stimulates the conversion of glucose carbon to $CO_2$, glyceride-glycerol, glyceride-fatty acids and glycogen in segments of epididymal fat [Jeanrenaud and Renold, 1959; Lynn et al., 1960]. With respect to the oxidation of glucose to $CO_2$, the stimulating effects of insulin on glucose carbon-1 are more pronounced than on glucose carbon-6 [Winegrad and Renold, 1958b], reflecting an increase in the oxidation by the phosphogluconate-oxidative pathway in adipocytes. Studies with isolated fat cells have shown that rat adipose tissue is regularly responsive to physiological concentrations of insulin in vitro (0.01-0.5 μu/ml) on the metabolism of glucose [Rodbell, 1964b; Goldrick, 1967a; Gries and Steinke, 1967; Gliemann, 1968].

Under basal conditions, adipose tissue derived from young rats metabolizes a greater proportion of radioactive glucose to $CO_2$ and total glycerides and a smaller amount to glycogen and lactic acid; 76% of radioactive glucose found in total glycerides is recovered in the
glycerol-fatty acid moiety and only 24% in the glyceride-glycerol fraction [Lynn et al., 1960]. This pattern of basal glucose metabolism is not altered by the stimulating action of insulin in vitro [Lynn et al., 1960], but it is modified by age and adiposity of the animals.

DiGirolamo and Mendlinger [1972] reported that in old rodents with fat cell enlargement the percentage incorporation of glucose-U-14C into CO₂ and glyceride-fatty acids was reduced, but the percentage incorporated into glyceride-glycerol was increased. The absolute quantity of glucose metabolized to CO₂ and total lipids per cell was similar over a wide range of adipose cell volume [DiGirolamo and Mendlinger, 1972]. Even though the pattern of basal glucose metabolism of enlarged rat adipocytes is unaffected by insulin [DiGirolamo et al., 1974], these cells are hyporesponsive to the addition of insulin in vitro as demonstrated in both the percentage increase over basal glucose metabolism as well as the absolute rates of glucose oxidation and triglyceride synthesis [Salans and Dougherty, 1971; DiGirolamo and Mendlinger, 1972]. These findings do not necessarily indicate that the lesser response to insulin of enlarged rat adipocytes represents a specific effect of cellular enlargement on insulin sensitivity, since the impaired effect of insulin in the cells derived from old and obese rats may reflect changes in the internal environment of the animals.

On the other hand, the dietary state of the animals influences the effects of insulin on glucose metabolism by adipose tissue in vitro. The large adipocytes obtained from rats fed ad lib have rates of insulin-stimulated conversion of glucose-1-14C to CO₂ and total lipids comparable with the small cells from fasted rats [Salans and Dougherty, 1971]. Furthermore, meal feeding with or without caloric restriction in rats can prevent the decline of fatty acid synthesis from glucose.
induced by aging, and can maintain the insulin responsiveness of adipose tissue [Goldrick et al., 1972a]. Studies have been performed on isolated cells from the same rat pad fat (graded by a differential flotation technique), where the effects of age, dietary factors and the internal environment of the animal are kept constant. They show that the enlarged adipocytes exhibit a greater rate of glucose conversion to CO₂ and triglycerides than do their smaller counterparts, under basal states and in the presence of insulin [Gries et al., 1974; Nielsen and Hansen, 1974]. This phenomenon is consistently found in large and small fat cells of each single specimen whether derived from fed and fasted rats [Gries et al., 1974] or from lean and obese rats [Nielsen and Hansen, 1974]. Nevertheless, both small and large adipose cells from fasted or obese rats show a reduction in their basal glucose metabolism and a diminution of response to added insulin, compared with cells of identical size from fed or lean rats [Gries et al., 1974; Nielsen and Hansen, 1974]. Therefore, insulin sensitivity of rat adipose tissue on glucose metabolism in vitro is affected by the adipose cell size and age as well as the dietary state and internal environment of the animals.

(b) Effects of Insulin on Glucose Metabolism in Human Adipose Tissue

Early studies with human adipose tissue showed low and inconsistent effects of insulin on glucose metabolism in vitro, with responses to only very high concentrations of insulin (10-1000 μU/ml). Several investigators had demonstrated that pharmacological concentrations of insulin stimulated both glucose uptake [Pozza et al., 1963] and intracellular metabolism of radioactive glucose, such as esterification of fatty acids, fatty acid synthesis, oxidation to CO₂ particularly via the phosphogluconate-oxidative pathway [Fessler and Beck, 1965; Fessler et
al., 1967] and glycogen synthesis [Davidson, 1972], in human subcutaneous or omental adipose tissue. However, in some investigations consistent effects of insulin on glucose metabolism could not be demonstrated [Hirsch and Goldrick, 1964; Björntorp, 1966]. The hyporesponsiveness of human adipose tissue to insulin in vitro, in the previous reports, seems to be methodological artifact rather than a real insensitivity to the action of the hormone. Experimental conditions, for example size of adipose tissue specimen and glucose concentrations in the incubation medium, as well as the age of the fat donor, affect the insulin sensitivity. It has been demonstrated that the effects of insulin on the metabolism of glucose to triglycerides or CO₂ in human adipose tissue diminish with a reduction in specimen size [Hirsch and Goldrick, 1964; Björntorp and Martinsson, 1967]. Thus, an increased proportion of damaged membrane to intact membrane of adipocytes, present in the smaller tissue shreds, may be responsible for the decreased action of insulin on glucose metabolism within the cells (which reflects a reduced glucose transport across the cell membrane).

Glucose concentrations in the incubation medium also influence basal glucose metabolism and its response to insulin. Under basal conditions, glucose at concentrations of 1.0-10.0 mM/l augments the incorporation of labelled glucose into glyceride-glycerol and CO₂. Conversely, in the presence of insulin, the stimulated triglyceride synthesis from glucose (L-1⁴C or U-¹⁴C) declines with a high level of glucose (5.0-10.0 mM/l) in the incubation medium, but glucose has no effect on the stimulation of glucose oxidation by insulin [Fessler et al., 1967; Björntorp and Martinsson, 1967; Goldrick, 1967a; Salans et al., 1974].

The insulin-stimulated utilization of glucose by adipose tissue of
humans is age-dependent. Adipose tissue removed from children aged
between 5-15 years shows a greater sensitivity to insulin on the trans­
formation of glucose to glyceride-glycerol and CO₂ than does the tissue
of adults [Gries and Steinke, 1967; Salans et al., 1968].

Under optimal and standardized incubation conditions, glucose meta­
bolism in human adipose is markedly enhanced by physiological concentra­
tions of insulin (0.01-0.5 mu/ml). Studies with fragments of subcutaneous
adipose tissue have shown the effects of insulin on glucose uptake
[Englhardt et al., 1971a] and on the utilization of glucose for ester­
ification of FFA for synthesis of glyceride-fatty acids or for formation
of CO₂ [Björntorp, 1967a; Owen et al., 1967; Gries and Steinke, 1967;
Salans et al., 1974] as well as for glycogen synthesis [Davidson, 1975].

Isolated human subcutaneous adipocytes are sensitive to even lower
concentrations of insulin. Only 0.002 mu/ml of the hormone induces a
significant rise in triglyceride formation from glucose-U-¹⁴C. The
dose-responsive curves of insulin concentrations between 0.002-0.5 mu/ml
for the synthesis of glycerides and CO₂ are sigmoid, with the maximal
stimulating concentration of insulin obtained at 0.1 mu/ml [Goldrick,
1967a]. However, both basal and insulin-induced fatty acid synthesis
from glucose were not observed, as well as the lack of insulin action on
the acceleration of glucose oxidation by the phosphogluconate-oxidative
pathway [Goldrick, 1967a]. This observation is divergent from those
recorded in isolated rat fat cells and partly reflects damage of the
plasma membranes which occurs during the preparation of free cells by a
collagenase treatment [Goldrick, 1967a; Salans et al., 1968]. The
artifact induced by the technique of cell preparation can be corrected
by using concentrated isolated human adipose cells and a prolonged
incubation (6 hours). Since the synthesis of glyceride-fatty acids from
glucose on basal conditions and on the addition of insulin 1.0 mu/ml (a slightly higher concentration than physiological values) has been demonstrated in these omental fat cells [Goldrick et al., 1969].

(i) Effect of insulin on the distribution of labelled glucose in adipose tissue glycerides

When human subcutaneous or omental adipose tissue were incubated with glucose-1-\(^{14}\)C under basal conditions, approximately 40% of recovered radioactive glucose was present in CO\(_2\) and 59% in total glycerides [Fessler and Beck, 1965]. The major portion of the incorporated glucose carbon in the total lipids was found in the glyceride-glycerol fraction and only 15% or 16% of labelled glucose was recovered in the glyceride-fatty acid moiety, as shown in studies with intact tissue segments [Goldrick and Hirsch, 1964; Gries and Steinke, 1967], or with isolated fat cells [Galton, 1968; Goldrick et al., 1969; Smith, 1971]. Furthermore, insulin did not alter the percentage distribution of glucose incorporated into lipid classes in vitro [Goldrick et al., 1969; Smith, 1971].

(ii) The role of adipose cell size on insulin sensitivity of adipose tissue

The basal glucose metabolism in human adipose tissue in vitro is directly proportional to adipose cell size (see "Metabolism of Human Adipose Tissue in vitro" in Section 1.1). Several investigators have shown that the effect of insulin on the conversion of glucose-1-\(^{14}\)C or glucose-U-\(^{13}\)C to glyceride-glycerol, glyceride-fatty acids and glycogen is not impaired in enlarged human fat cells [Salans et al., 1968; Goldrick and McLoughlin, 1970; Davidson, 1972; Salans et al., 1974]. Furthermore, the number of insulin binding sites and
affinity to insulin in large human fat cells are the same as in small cells [Amatruda et al., 1974, 1975]. An impaired effect of insulin *in vitro* on the oxidation of glucose-1-^{14}C to CO_{2} with adipose cell enlargement from obese subjects was first observed by Salans *et al.* [1968]. This observation has been confirmed in another study with adipose tissue from overweight subjects and subjects following experimentally induced adipose cell enlargement [Salans *et al.*, 1974]. Smith [1971] also reported a slight reduction in the percentage increase over the basal rate of triglyceride synthesis from glucose in the presence of insulin in enlarged human adipocytes; however, the absolute basal and insulin-stimulated rates of glucose metabolism were markedly increased in large cells. It is unlikely that such cells are insulin resistant. Therefore, in studies with human adipose tissue *in vitro*, the enlarged fat cells exhibit resistance to the effects of insulin only on the oxidation of glucose-1-^{14}C to CO_{2}, measuring only one pathway of glucose metabolism (the phosphogluconate-oxidative shunt).

The hyporesponsiveness of large human adipocytes to the effect of insulin *in vitro*, on this particularly sensitive parameter of glucose metabolism, possibly reflects a residual effect of changes in the internal environment of the adipose tissue donor [Nestel and Goldrick, 1976], not a consequence of cellular enlargement.

(iii) Effects of dietary changes on insulin responsiveness of adipose tissue

The effect on insulin on glucose metabolism of rat adipose tissue *in vitro* is altered by the nutritional and metabolic status of the animals, as has already been discussed. At present, there is substantial evidence to indicate that various dietary manipulations in
human subjects modify the effect of insulin \textit{in vitro} on glucose metabolism in adipose tissue. A caloric-restricted diet of 900 calories/day, administered to obese subjects for 12-16 days, resulted in inhibition of the stimulating effects of insulin (0.625 µu/ml) on the utilization of glucose-1-\textsuperscript{14}C for oxidation to CO\textsubscript{2} and for esterification of fatty acids by subcutaneous fat [Bray, 1969]. Moreover, the same source of tissue removed from obese subjects on a hypocaloric diet of 500 calories/day for 2-7 days showed a decreased rate of glucose-U-\textsuperscript{13}C metabolization into CO\textsubscript{2} and glycogen in response to insulin (0.1 and 1.0 µu/ml). This was in comparison with tissue obtained from obese and non-obese subjects who consumed a weight-maintaining diet [Davidson, 1975].

High carbohydrate diets have been shown to enhance the effects of insulin on glucose metabolism of human adipose tissue. Isocaloric substitution of carbohydrate for dietary fat in obese subjects resulted in stimulation of triglyceride synthesis from glucose by adipose tissue, incubated with 0.1 µu/ml of insulin \textit{in vitro} [Hirsch and Goldrick, 1964]. Moreover, feeding an isocaloric high sucrose diet for 14 days to hypertriglyceridaemic subjects enhanced the stimulating effect of insulin (0.01 µu/ml) on triglyceride synthesis from glucose [Smith \textit{et al.}, 1973]. Furthermore, in an elegant study of Salans \textit{et al.} [1974], isocaloric high or low carbohydrate diet was alternately administered for 21-day periods to normal subjects (during the control period and after the experimentally induced weight gain, and to obese subjects (during the weight-maintaining period and after weight reduction). It appears that whilst the high carbohydrate diet increases the responsiveness to physiological doses of insulin on the conversion of glucose-1-\textsuperscript{14}C to CO\textsubscript{2} and glyceride-glycerol regardless of the adipose cell size, the low carbohydrate diet has the reverse effect. In their study, basal
incorporation of glucose to glyceride-fatty acids could not be demonstrated during any of the dietary manipulations [Salans et al., 1974]. A hypercaloric, high carbohydrate diet given to obese subjects for 5 days accentuated the insulin-mediated synthesis of glyceride-glycerol from glucose in adipose tissue, but did not alter the action of insulin on the conversion of glucose to fatty acids, despite an increased activity of enzymes for de novo fatty acid synthesis in the tissue [Sjöström, 1973b].

There is relatively less evidence concerning the effects of dietary fat on insulin stimulation of glucose metabolism in human adipose tissue. Sims et al. [1973] found that an isocaloric exchange of dietary fat for carbohydrate impaired the insulin effects in vitro on glucose oxidation and pyruvate lipogenesis in experimentally induced obese subjects.

On the other hand, feeding a high fat diet in rats caused a decreased insulin sensitivity of adipose tissue on the metabolism of glucose to CO₂, glyceride-glycerol and glyceride-fatty acids [Ogundipe and Bray, 1974].

Adult overweight subjects, varying in their body weights over a wide range of adipose cell size, were administered a control diet providing 37% of calories as carbohydrate for 3 days. The oxidation of glucose-1⁻¹⁴C to CO₂, in subcutaneous adipose tissue pieces incubated without or with insulin (1.0 μu/ml), was positively correlated with the mean cell size, independent of age and relative body weight [Harrison and King-Roach, 1976]. This observation is in accordance with the previous study of Davidson [1975], who has shown that the maximal stimulating effect of insulin on the increased incorporation of glucose to CO₂ over the basal value, in segments of subcutaneous adipose tissue
from obese subjects, is significantly greater than that recorded in adipose tissue from normal weight subjects.

The sensitivity of human adipose tissue to insulin in vitro reflects both the nutritional state and the adipose cell size of the subjects. Dietary factors seem to exert a more important effect on insulin sensitivity than adipocyte size; Salans et al. [1974] have reported that the per cent contribution of dietary factors to the total variability of insulin stimulated glucose oxidation and esterification of fatty acids in human adipose tissue is greater than that of cell size.

In summary, glucose metabolism in human adipose tissue in vitro is potentiated by physiological concentrations of insulin similar to those measured in plasma after glucose ingestion. The insulin effects are dependent on the technique of incubation, the age of the adipose tissue donor, adipose cell size and in particular the antecedent dietary manipulations and metabolic states of the subjects before removal of adipose tissue samples.

1.4 HORMONAL CONTROLS ON LIPID MOBILIZATION IN HUMAN ADIPOSE TISSUE IN VITRO

Mobilization of FFA from adipose tissue is achieved by breakdown of triglycerides in adipocytes. The lipolytic pathway and its activators as well as the antilipolytic effects of insulin and other substances, as studied in adipose tissue in vitro, will be discussed as follows:

(a) Lipolytic Pathway

Human adipocytes respond to lipolytic hormones by an increase in the accumulation of intracellular cyclic AMP via activation of adenyl
cyclase, which stimulates a protein kinase. The latter in turn phosphorylates a triglyceride lipase resulting in hydrolysis of triglycerides to glycerol and FFA.

Studies using pieces of human adipose tissue or isolated fat cells have demonstrated the lipolytic effects of catecholamines or theophylline or dibutyryl cyclic AMP, as judged by increments of glycerol or FFA production [Burns and Langley, 1968; Gries et al., 1968; Moskowitz and Fain, 1969; Östman et al., 1969; Efendic and Östman, 1970; Östman et al., 1973]. Glucagon (12.5 \mu g/ml) also induces an increase in glycerol release from isolated human subcutaneous fat cells [Björntorp et al., 1969b]. It should be mentioned that in most studies, glycerol release is taken as an accurate index of lipolysis, since it is not readily re-utilized by adipose tissue [Björntorp, 1967b].

Catecholamine-induced lipolysis is initiated by interaction between the hormones and adrenergic receptors on fat cell membranes. Addition of adrenergic stimulating or blocking agents showed the existence of beta- as well as alpha-adrenergic receptors on the surface of human fat cells [Burns and Langley, 1970; Östman and Efendic, 1970]. Lipolysis induced by epinephrine or norepinephrine (mixed-adrenergic stimulators) or isopropylnorepinephrine (a predominantly beta-adrenergic stimulant) was completely suppressed by a beta-adrenergic blocker, propanolol. The latter agent had no influence on basal lipolysis, or lipolysis stimulated by theophylline. Furthermore, norepinephrine-induced lipolysis was potentiated by the addition of low concentrations of an alpha-adrenergic blocking agent, phentolamine (0.05-100 \mu g/ml). Such concentrations did not alter the lipolytic effects of isopropylnorepinephrine or theophylline [Burns and Langley, 1970; Östman and Efendic, 1970]. Therefore, in response to catecholamines of human adipose tissue
the beta-adrenergic receptors accelerate lipolysis, which is counter-
acted by a simultaneous stimulation of the alpha-adrenergic receptors
(resulting in an attenuation of catecholamine-induced lipolysis).

As a result of the interaction, adenyl cyclase is activated. In
recent studies, homogenates of adipose tissue [Kissebah and Fraser,
1972], or fat cell membranes ("ghosts" [Burns et al., 1975]) possess
adenyl cyclase (a membrane-bound enzyme) activity, which is measured as
the generation of cyclic AMP from labelled ATP. Moreover, this adenyl
cyclase activity is potentiated by the maximal stimulation of epine-
phrine (1 × 10^-5 M/l) or by sodium fluoride (10 mM/l), a non-specific
activator of adenyl cyclase [Burns et al., 1975].

Catecholamine-induced lipolysis in vitro is associated with a
raised level of adipose tissue cyclic AMP prior to an increase in
glycerol release [Kissebah and Fraser, 1972; Gilbert and Galton, 1974;
Arner, 1976]. Furthermore, human adipose tissue pieces respond to sub-
maximal (1 × 10^-7 M/l) or maximal (6 × 10^-5 M/l) effective dosages of
catecholamines by a prompt rise in tissue cyclic AMP which reaches a
peak within 10 minutes and returns to basal levels in 1 hour [Gilbert
and Galton, 1974; Arner, 1976]. On the other hand, the rate of
glycerol release is a linear function of time, up to 4 hours. This
indicates that phosphorylation of the intracellular triglyceride lipase
and the subsequent breakdown of triglycerides can be maintained without
a constant elevation of tissue cyclic AMP [Arner, 1976].

Essential enzymes involved in the breakdown of triglycerides have
been identified in human adipose tissue. One study using homogenates of
adipose tissue demonstrated the presence of a cyclic AMP-dependent
protein kinase. Its activity, determined by measuring the
phosphorylated rate of histone by γ-32P-ATP, is potentiated by
exogenous cyclic AMP [Kissebah et al., 1974b]. Moreover, adipose tissue homogenates have a triglyceride lipase (a rate-limiting enzyme in lipolysis) activity [Björntorp, 1967b]. Recently, this enzyme has been partially purified, and its lipolytic activity is stimulated by the addition of cyclic AMP, ATP and a purified protein kinase [Kissebah et al., 1974b; Khoo et al., 1974].

The presence of monoglyceride and diglyceride lipases were also demonstrated in homogenates [Björntorp, 1967b; Khoo et al., 1974] or in acetone-ether extracts [Elkeles, 1974] of adipose tissue. Moreover, the diglyceride lipase is also shown to be a rate-limiting enzyme in lipolysis [Arner et al., 1976]. A cyclic AMP-phosphodiesterase activity, as measured by the transformation of cyclic AMP-3H to 5-AMP-3H, is present in human subcutaneous adipose tissue [Kissebah and Fraser, 1972]. This enzyme acts by degrading cyclic AMP into its non-cyclic derivative, leading to an inhibition of lipolysis in rat adipose tissue [Butcher and Sutherland, 1962].

It is, therefore, possible to conclude that the reaction chain leading to the hydrolysis of triglycerides is demonstrable in human adipose tissue. Catecholamines induce lipolysis by stereospecific binding to the beta-adrenergic receptors on the adipocyte membrane. Glucagon also binds to a receptor on the cell surface. These lipolytic hormones interact with adenyl cyclase. This interaction brings about an increase in the transformation of ATP to cyclic AMP, which in turn activates the cyclic AMP-dependent protein kinase resulting in a phosphorylation of triglyceride lipase. The active triglyceride lipase hydrolyzes triglycerides into partial glycerides and FFA. The di- and monoglyceride lipases further degrade the partial glycerides to glycerol and FFA. In addition, the activation of triglyceride lipase can be
mediated by an inhibiting effect of theophylline on cyclic AMP-phosphodiesterase or by a stimulating action of dibutyryl cyclic AMP.

Increased lipolysis in adipose tissue induced by negative caloric balance does not seem to be modulated by increased tissue levels of cyclic AMP. A 10-day period of fasting in obese subjects led to an increased production of glycerol from adipose tissue in vitro without any changes in the concentration of tissue cyclic AMP [Gilbert and Galton, 1974]. Therefore, the synthesis of new triglyceride lipase during caloric deprivation is probably responsible for the increased basal lipolytic activity, since the magnitude of responsiveness of adipose tissue to isopropylnorepinephrine recorded at a pre-fasting period is similar to the result of a post-fasting state [Gilbert and Galton, 1974]. This observation indicates the same ratio of inactive to active lipase in the adipocytes of both states [Gilbert and Galton, 1974].

(i) Influence of glucose concentrations

Glucose at concentrations of 1.0 or 11.0 mM/l produces a significant rise of glycerol production and a significant fall of FFA release from human adipose tissue, but has no influence on tissue FFA concentration [Smith, 1970b; Efendic and Östman, 1970]. This stimulating effect of glucose on glycerol release is better observed in the presence of catecholamines or dibutyryl cyclic AMP. Moreover, addition of glucose results in a further rise of tissue FFA concentration induced by norepinephrine, without significant change in the rate of FFA release [Efendic and Östman, 1970].

In rat adipose tissue, glucose potentiates glycerol release induced by catecholamines by 2 different mechanisms. Glucose reduces both tissue FFA concentration and FFA release, presumably by supply of alpha-
glycerophosphate for the re-esterification of FFA [Jungas and Ball, 1963]. The reduction in tissue FFA (an inhibitor of lipolysis) to an appropriate level causes a further stimulation on lipolysis, as judged by the glycerol output [Bally et al., 1965]. The other mechanism is that glucose maintains levels of tissue ATP, during the lipolytic process induced by catecholamines. Since ATP is required for the formation of cyclic AMP and in the phosphorylation of triglyceride lipase by a protein kinase, glucose can enhance glycerol release with no reduction in the concentration of tissue FFA or the release of FFA [Ho et al., 1970]. Thus, findings in human adipose tissue on glucose stimulation of lipolysis induced by the hormones favour with the second possibility, observed in rat adipose tissue. The increase in fat breakdown by glucose in vitro is due to an improved energy metabolism in the cells. The first mechanism probably operates on non-stimulated lipolysis in human adipose tissue.

(ii) Adipose cell size and lipolysis

Studies with both fragments and isolated cells of human adipose tissue have shown that basal lipolysis and the absolute stimulating effects of catecholamines, theophylline, or dibutyryl cyclic AMP are directly proportional to the size of the fat cells. These data were derived from experiments performed in the presence or absence of added glucose and were expressed as rate of lipolysis per cell [Goldrick and McLoughlin, 1970; Knittle and Ginsberg-Fellner, 1972; Jacobsson and Smith, 1972; Gries et al., 1972; Östman et al., 1973].

However, the increase of glycerol formation above base line values induced by these lipolytic agents is not correlated to the fat cell size. In the presence of epinephrine or norepinephrine, the changes in
lipolysis over the basal values are comparable in both small and large cells [Knittle and Ginsberg-Fellner, 1972; Östman et al., 1973] or are decreased with increasing the size of the fat cells [Goldrick and McLoughlin, 1970; Gries et al., 1972]. Gries et al. [1972] have suggested that enlarged adipocytes are associated with increases in total lipase activity and in the proportion of active lipase to inactive enzyme. The reduction in relative stimulation by the lipolytic agents in large fat cells is due to the raised basal lipolysis [Gries et al., 1972].

(b) The Antilipolytic Effects of Insulin

Lipolytic activity of human adipose tissue in vitro is reduced by insulin in the absence of glucose, as shown by a reduction in glycerol release. With tissue fragments, a significant inhibition of lipolysis by physiological concentrations of insulin (0.001-0.1 μu/ml) was demonstrated both on basal and catecholamine-induced lipolysis [Gries et al., 1968; Östman et al., 1971; Östman et al., 1975]. In the presence of glucose in the incubation medium, the antilipolytic effects of insulin on the basal lipolysis was observed [Björntorp and Hood, 1966]. However, in another study, the basal glycerol release and that stimulated by epinephrine and isopropylnorepinephrine were inconsistently inhibited by insulin [Jacobsson and Smith, 1972]. Insulin exerted a consistent inhibition on lipolysis, only in the presence of norepinephrine or salbutamol (a beta adrenergic agonist) [Jacobsson and Smith, 1972]. On the other hand, insulin has inhibiting effects in isolated cells studied in the presence or absence of added glucose, where the rate of basal lipolysis is high, for example in diabetes mellitus or in clinical conditions associated with severe
stress [Lisch et al., 1974], or when lipolysis is provoked by epinephrine, norepinephrine or theophylline [Burns and Langley, 1968; Moskowitz and Fain, 1969; Goldrick and McLoughlin, 1970]. Therefore, insulin at physiological dosages seems to consistently inhibit the elevated glycerol production in human adipose tissue *in vitro*, irrespective of the presence of glucose.

The mediation of insulin action to inhibit lipolysis is probably via its cyclic AMP-lowering effect. Insulin prevents the increase in intracellular cyclic AMP in human adipose tissue exposed to norepinephrine [Kissebah and Fraser, 1972] or isopropynorepinephrine [Gilbert and Galton, 1974], resulting in the suppression of lipolysis. Insulin may act on other levels in the lipolytic pathway, which have already been discussed in the context of rat adipose tissue (see "Actions of Insulin on Adipose Tissue" in Section 1.2). However, no data are available to support such an effect in human adipose tissue, except for the inhibition of theophylline-induced lipolysis by insulin, reflecting its stimulating action on cyclic AMP-phosphodiesterase. The mechanism by which insulin also reduces basal lipolysis in human adipose tissue pieces remains unclear; perhaps such tissue preparation still contains its endogenous catecholamines.

It should be noted that insulin at pharmacological concentrations (10-100 μg/ml) fails to suppress the lipolytic effects of catecholamines [Jacobsson and Smith, 1972; Gilbert and Galton, 1974], both in glucose-free and glucose-containing medium.

Insulin inhibits catecholamine-stimulated lipolysis to an equal extent in small and large fat cells, as judged by the absolute rate of glycerol production [Goldrick and McLoughlin, 1970; Knittle and Ginsberg-Fellner, 1972]; This insulin-induced inhibition of
catecholamine-mediated lipolysis may even be greater in the large fat cells [Östman et al., 1975]. However, the per cent reduction of hormone-stimulated glycerol release in the presence of insulin is lower if enlarged adipocytes obtained from obese subjects [Faulhaber et al., 1970; Gries, 1970]. It is unlikely that enlarged adipocytes are less sensitive to inhibitory effects of insulin. The apparent hypo-responsiveness of enlarged adipocytes to insulin is due to a reduction in the relative stimulation, induced by catecholamines, over the baseline.

(c) Other Antilipolytic Agents

Besides insulin, other agents have been shown to possess antilipolytic effects on human adipose tissue *in vitro*. Prostaglandins, especially E₁ (PGE₁) have inhibitory effects on lipolysis induced by norepinephrine, isopropynorepinephrine or theophylline, but have no influence on basal or dibutyryl cyclic AMP-stimulated lipolysis [Carlson and Hallberg, 1968; Moskowitz and Fain, 1969; Efendić, 1970; Gilbert and Galton, 1974]. It has been proposed that PGE₁ interferes with lipolysis at the level of adenyl cyclase, so it inhibits the formation of tissue cyclic AMP [Moskowitz and Fain, 1969]. This is supported by a report of Gilbert and Galton [1974] that PGE₁ reduced concentration of tissue cyclic AMP in the presence of catecholamines. Moreover, Efendić [1970] has reported that PGE₁ acts by inhibiting both beta- and alpha-adrenergic effects of norepinephrine, since its inhibitory action on norepinephrine-induced glycerol production is augmented by the addition of phentolamine (50 μg/ml). *High concentration of phentolamine* (500 μg/ml) reduced both norepinephrine- and theophylline-mediated lipolysis, therefore, its antilipolytic effect is situated between the
formation of cyclic AMP and the activation of triglyceride lipase [Üstman and Efendić, 1970]. Studies in rat adipose tissue, nicotinic acid has been shown to suppress the action of catecholamines on the formation of cyclic AMP via its inhibition of adenyl cyclase activity [Fain, 1973]. An intravenous injection of nicotinic acid in human subjects leads to a significant fall in plasma FFA [Schlierf and Dorow, 1973; Tasaka et al., 1976], reflecting its antilipolytic action in adipose tissue.

Increased accumulation of FFA in isolated human fat cells during a prolonged incubation or addition of sodium oleate of high concentrations (2-4 mM/l) appears to interfere with the lipolytic activity induced by isopropylnorepinephrine [Burns et al., 1975]. The inhibitory effect of FFA on lipolysis is probably localized at the level of adenyl cyclase, as shown by a subsequent reduction of tissue cyclic AMP [Burns et al., 1975].

In conclusion, human adipose tissue studied in vitro possesses a lipolytic system which is regulated by a number of hormones and a variety of agents; catecholamines and other lipolytic agents are responsible for acceleration of lipolysis which is antagonized by the action of insulin and other antilipolytic agents including beta-adrenolytic agents and high concentration of alpha-adrenolytic agents.

It is well documented that the mobilization of FFA from adipose tissue for use as a metabolic fuel is mainly governed by lipolysis and to a lesser extent by changes in the rate of re-esterification of FFA. In vivo, lipolysis can be induced by an increased activity of the sympathetic nervous system, mediated through the effect of catecholamines by the mechanism that has been observed in studies in vitro. Even though breakdown of fat can be inhibited by several agents in
vitro, their physiological significance remains controversial. Only insulin at physiological concentrations suppresses elevated plasma FFA, presumably reflecting an increase in esterification of FFA and an inhibition of lipolysis in adipose tissue.

1.5 INSULIN SECRETION

Insulin is synthesized in the beta cells of pancreas as a single chain polypeptide precursor, proinsulin, which is then enzymatically converted to insulin molecule [Steiner and Oyer, 1967; Kemmler and Steiner, 1970]. Howell et al. [1969] have shown that in the beta cells of rat islets of Langerhans, labelled amino acids are incorporated into the rough surfaced endoplasmic reticulum. They are then transported into the Golgi region, and are packaged into the insulin secretory granules. Insulin is secreted from the human beta cells in response to various insulin secretagogues. Among them, glucose is a primary and potent stimulus, as demonstrated in studies with animal pancreas perfused or incubated with glucose [Grodsky et al., 1963; Coore and Randle, 1964]. Insulin secretion in response to glucose is possibly brought about by an accumulation of intracellular cyclic AMP [Charles et al., 1973; Grill and Cerasi, 1974]. The latter substance has been shown to be an essential activator of insulin release from pancreatic islets [see review by Donnelly and Turtle, 1974]. Recently, studies with isolated rat pancreatic islets have shown that glucose added in vitro rapidly increases the accumulation of tissue cyclic AMP [Grill and Cerasi, 1974]. Moreover, there is a correlation between the release of insulin and cyclic AMP from islet preparation into the incubation medium [Grill and Cerasi, 1974]. Cerasi [1975] has suggested that cyclic AMP is perhaps a mediator for glucose action to the site of insulin release in the beta
cells. Moreover, cyclic AMP may provoke an efflux of mitochondrial calcium [Brisson et al., 1971], which in turn enhances the release of insulin from the beta cells [Cerasi, 1975]. It is proposed that glucose stimulates insulin synthesis in rat islets [Curry, 1970]. This is supported by an increase in ribonucleic acid (RNA) content of the beta cells in the presence of glucose [Jarrett et al., 1967]. However, the exact mode of glucose action on insulin secretory mechanism remains in doubt.

Gastrointestinal hormones also provoke insulin release from human pancreas. The hormonal factors secreted from the gastrointestinal tract during an oral glucose load account for an induction of higher plasma insulin concentration, as compared with that recorded after intravenously administered glucose [McIntyre et al., 1964; Dupré, 1964; Perley and Kipnis, 1967]. Many preparations of enteric hormones possess insulinotrophic effects in man, for example gastrin [Rehfeld and Stadil, 1973], secretin [Dupré et al., 1966; Chisholm et al., 1969; Kraegen et al., 1970], glucagon-like immunoreactivity [Samols et al., 1965] and gastric inhibitory peptide (GIP) [Dupré et al., 1973]. The insulin releasing effect of secretin and GIP may be of some physiological significance, since an oral glucose load induces a rise in plasma concentrations of secretin [Chisholm et al., 1969] or GIP [Cataland et al., 1974]. However, the mechanism whereby these agents increase insulin release from the beta cells is uncertain, possibly they have a direct stimulating action on the beta cells or perhaps they only potentiate the effect of glucose. A more recent study supports the latter concept, since the rise in plasma secretin, induced by gastric administration of 200 ml 0.1 M HCl to normal subjects in the post-absorptive state, does not significantly alter levels of plasma glucose.
or insulin [Schaffalitzky de Muckadell et al., 1976].

Administration of medium-chain or long-chain triglycerides results in a significant rise in serum insulin [Pi-Sunyer et al., 1969; Carroll and Nestel, 1972]. A rise in plasma ketones accompanying the ingestion of the medium-chain triglycerides is a cause of increased insulin secretion [Pi-Sunyer et al., 1969]. The long-chain triglycerides in the high fat meal increase insulin production without changes in total blood ketone levels, thus their fat-induced gastrointestinal hormone release is probably the reason for elevated plasma insulin [Pi-Sunyer et al., 1969; Carroll and Nestel, 1972].

The other insulin releasers in humans are proteins and amino acids [Fajans et al., 1967; Floyd et al., 1966], and glucagon [Crockford et al., 1966; Schade and Eaton, 1976], as well as sulfonylureas [Cerasi et al., 1969; Windström and Cerasi, 1973].

Insulin is secreted from at least two functional pools within the beta cell. Perfusion of isolated rat pancreas by glucose has shown that the pattern of insulin secretion is biphasic, indicating two separate compartments of insulin storage [Curry et al., 1968; Curry, 1970]. In response to glucose stimulus, the early phase of insulin production lasts about 2 minutes, representing a rapid release of the performed hormone. The quantity of insulin secreted from this small releasable pool is unaltered by puromycin (an inhibitor of protein synthesis). The late phase of insulin secretion is derived from a larger secretory pool. This pool is composed of both the stored and newly synthesized insulin, since isolated pancreas preincubated with puromycin has a reduction in the second phase of insulin release [Curry et al., 1968; Curry, 1970]. Evidence of the multiphasic pattern of insulin secretion induced by glucose stimulus has been demonstrated in
normal subjects [Cerasi and Luft, 1967; Porte and Pupo, 1969; Porte and Bagdade, 1970]. Furthermore, relative acute insulin response above the fasting level is significantly correlated with the glucose disappearance rate (Kg). This was observed after a rapid injection of 20 g glucose intravenously to subjects with fasting plasma glucose below 115 mg/100 ml [Brunzell et al., 1976]. Additionally, the absolute incremental insulin secretion during the initial phase is proportional to the dosage of glucose injected [Pelkonen et al., 1974]. This labile compartment of insulin within the beta cells is available for release in response to acute elevation of blood glucose concentration [Lerner and Porte, 1971; Pelkonen et al., 1974]. A decrease or lack of rapid insulin secretion following an intravenous glucous load in subjects with carbohydrate intolerance [Cerasi and Luft, 1967; Seltzer et al., 1967; Lerner and Porte, 1972; Brunzell et al., 1976] supports the physiological importance of this small, readily available pool in regulation of the rate of glucose utilization. The slowly reacting pool for the late phase of insulin secretion is probably related to metabolism of glucose and rate of insulin synthesis [Porte and Pupo, 1969; Porte and Bagdade, 1970].

Normal subjects respond to an intravenous glucose tolerance test (GTT) with a rapid rise of plasma insulin. Insulin reaches maximal levels within 5 minutes, and there is a decline to near fasting levels by 1 hour [Seltzer et al., 1967]. Whilst insulin secretion during an oral GTT in normal individuals is shown as a sustained increase with a peak at 45 minutes, it gradually decreases to the level observed at postabsorption by 4 hours [Seltzer et al., 1967]. On the other hand, obesity is usually associated with hyperinsulinaemia. This phenomenon has been observed under basal states [Bagdade et al., 1967; Seltzer et
or following stimulation by the insulin secretagogues, for example glucose [Karam et al., 1963; Seltzer et al., 1967; Pelkonen et al., 1974; Karam et al., 1974], tolbutamide [Perley and Kipnis, 1966; Kreisberg et al., 1967], glucagon [Benedetti et al., 1967; Crockford et al., 1969; Solter et al., 1976] and amino acids [Loridan et al., 1971; Johnson et al., 1973]. As a consequence of an increase in insulin secretory units of the pancreatic islets in obesity, both early and late phase of insulin response to glucose infused intravenously are more pronounced than those recorded in normal weight subjects [Pelkonen et al., 1974; Karam et al., 1974]. It has been suggested that hyperinsulin secretion of overweight subjects is due to a greater amount of insulin in the store available for release, not an increase in sensitivity of the beta cells to stimulators [Pelkonen et al., 1974; Karam et al., 1974].

In diabetic patients without associated obesity, as mentioned above, there is a reduction or absence of the first phase of insulin secretion induced by intravenous glucose load. Following glucose ingestion mildly diabetic subjects have a delayed hyperinsulinaemia following a slow, initial rise of insulin, indicating an impairment in early insulin secretory response to stimuli [Perley and Kipnis, 1967; Seltzer et al., 1967]. This phenomenon leads to a prolonged hyperglycemia, reflecting a slow removal of circulating glucose to serve as a late stimulus to the beta cells [Perley and Kipnis, 1967; Seltzer et al., 1967]. In contrast, the insulin responsive curve observed in moderate diabetics is low following glucose ingestion despite a higher degree of hyperglycemia [Seltzer et al., 1967].

When obese subjects have associated mild diabetes, their hyperinsulinaemia induced by oral glucose still persists [Bagdade et al.,
but the initial phase of insulin secretion in response to acute glucose load is impaired [Luft et al., 1968]. Severely diabetic, obese subjects have shown a reduction of insulin release following GTT similar to that occurring in severely diabetic, non-obese subjects [Bagdade et al., 1974], indicating an impairment in functional capacity of the pancreas.

In summary, the secretion of insulin from the beta cell in response to intravenous glucose administration is biphasic. The first phase or acute insulin response is associated with the hormone stored in a small readily available pool. The second phase or late insulin response is derived from a larger and more slowly reacting pool. An excessive insulin response to glucose is usually found in obesity. There is a reduced or lack of the initial insulin response to glucose in the mildly diabetic state. Insulin secretory pattern of mildly diabetic subjects (following an oral glucose load) is characterized by a delayed rise with prolonged hyperinsulinaemia. Whilst a hypoinsulin response to glucose is shown in moderate or severe diabetes mellitus. Obese diabetics show an insulin secretory response to glucose stimulus in the same manner as non-obese diabetics.

1.6 ROLE OF THE LIVER IN REGULATION OF BLOOD GLUCOSE HOMEOSTASIS IN HUMANS

The liver is capable of synthesizing glycogen, lipids, and proteins, which are converted in part to glucose and ketoacids, for the use in metabolism of other tissues. Glucose is an obligatory substrate for oxidative processes in brain and nervous tissue [Scheinberg, 1965] as well as red blood cells [Murphy, 1960]. Both nutritional and hormonal states determine metabolic activities in the liver. The passage of
glucose across the liver cell membrane is not a rate-limiting step [Cahill et al., 1959]. In the postabsorptive state, splanchnic glucose production is derived from gluconeogenesis and glycogenolysis in the liver [Cahill et al., 1970]. Gluconeogenic substrates are lactate and pyruvate from metabolism in muscle or blood cells, glycerol from lipolysis in adipose tissue, and amino acids (principally alanine [Felig et al., 1969]) from breakdown of muscle proteins [Cahill et al., 1970]. Hepatic glycogenolysis provides glucose for emergency supply [Williams and Ensinck, 1966]. Hepatic gluconeogenesis may be the more important regulatory mechanism, which maintains blood glucose level during brief fasting (3-4 days), since glycogen stored in human liver was almost used in the postabsorptive state and was scarcely detectable after 24 hours of complete starvation [Nilsson and Hultman, 1973].

(a) Hormonal Controls

The following hormones, insulin, glucagon, catecholamines, growth hormone and glucocorticoids exert their actions, directly or indirectly, on carbohydrate metabolism in the liver.

(i) Insulin

The actions of insulin on liver metabolism have been reviewed [Williams and Ensinck, 1966], in particular with respect to glucose metabolism, hepatic glucose intake and output. In summary:

(a) insulin stimulates the activities of the key glycolytic enzymes, viz., glucokinase (an important rate-limiting enzyme in the first step of glucose phosphorylation), phosphofructokinase and puruvate kinase.
(b) insulin promotes glycogen synthesis in the liver by activating glycogen synthetase (UDPG-glycogen glucosyltransferase). Inhibition of hepatic glycogenolysis is also the effect of insulin, mediated by its cyclic AMP-lowering effect.

(c) insulin facilitates hepatic lipogenesis by its stimulating effects on the key enzymes of fatty acid synthesis.

(d) insulin depresses activities of the key gluconeogenic enzymes (pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase and glucose-6-phosphatase).

Therefore the overall effect of insulin on carbohydrate metabolism in the liver is to increase the utilization of glucose, resulting in increased glucose uptake, and decreased glucose output [Williams and Ensinck, 1966].

In normal man during postabsorption, hepatic glucose output is approximately 180 g per day, as studied by hepatic venous catheterization [Myers, 1950]. Felig and Wahren [1971] used brachial arterial and hepatic venous catheter techniques in normal postabsorptive subjects to show that the liver releases glucose and takes up lactate and pyruvate as well as amino acids, predominantly alanine. This quantity of glucose is mostly consumed by the central nervous system, blood cells, and to a lesser extent by muscle and other tissues [Cahill et al., 1970; Felig et al., 1975].

Liver plays an important role in controlling glucose homeostasis, in so far as it has a relatively greater capacity in the disposal of an oral glucose load than peripheral tissues [Wahren and Felig, 1974; Felig et al., 1975]. Three hours after the ingestion of 100 g glucose, the splanchnic extraction of glucose is 60 g. Twenty five grams of
glucose leaving the splanchnic tissues are utilized by brain and blood cells. Only 15 g of ingested glucose load are delivered to peripheral tissues [Felig et al., 1975]. Moreover, raised concentrations of arterial lactate and pyruvate (of the order of 60-85%) after glucose loading were observed [Wahren and Felig, 1974]. The increased level of arterial lactate was the result of an inhibition of splanchnic uptake of lactate; the elevated arterial concentration of pyruvate was a consequence of the reversal of net pyruvate exchange from net uptake in the basal state to net production after glucose loading [Wahren and Felig, 1974]. In addition, forearm glucose uptake following an oral glucose tolerance test in normal subjects is only 42% of 100 g glucose administered [Jackson et al., 1973a]. The rise and fall of plasma lactate during the ingestion of glucose are not determined by the uptake and release of lactate by the forearm tissue [Jackson et al., 1973a]. From these observations therefore, the liver is a major site for the disposal of a carbohydrate load and it also determines the changes in blood lactate and pyruvate concentrations during the absorption of glucose.

It has been demonstrated in man that an infusion of exogenous insulin inhibits hepatic glucose production [Craig et al., 1961]. In a more recent study, Felig and Wahren [1971] have shown that liver is sensitive to a small increase in endogenous insulin concentration. A two-fold increase in arterial insulin level, induced by a low dose of glucose infusion (2 mg/kg/minute, for 45 minutes) into normal weight subjects, causes a decrease in splanchnic glucose output by 85%. This event occurs without alterations in the rate of hepatic gluconeogenesis or of peripheral glucose disposal. With a higher dosage of glucose infusion (25 mg/kg/minute, for 20 minutes) which produces a five-fold
rise in arterial insulin, net hepatic glucose uptake is noted. The reduction in gluconeogenesis is a consequence of the 30-60% fall of splanchnic uptake of gluconeogenic substrates (amino acids, pyruvate and lactate). Changes in arterial concentrations of these substrates were not observed during this high dose of glucose infusion. From the above findings, Felig and Wahren [1971] concluded that in man the liver responds to a small increase in endogenous insulin level by decreasing its glucose output. In response to a high level of insulin, liver takes up glucose and there is reduced extraction of circulating gluconeogenic substrates. Liver is therefore a more important determinant in the utilization of carbohydrate than other peripheral tissues, probably due to the higher concentration of insulin in the portal circulation [Felig and Wahren, 1971]. On the other hand, hepatic resistance to insulin effects was demonstrated in obese subjects in the basal state or in response to an intravenous glucose load. Felig et al. [1974] have noted that obesity with basal arterial hyperinsulinaemia (2-3 fold higher than in normal subjects) is associated with an increased splanchnic uptake of glucose precursors, particularly alanine and lactate. Basal gluconeogenesis accounts for 33% of hepatic glucose production in the obese subjects, as compared with 19% in controls. Furthermore, a higher level of glucose infusion is required to produce a comparable inhibition in splanchnic glucose output to that observed in normal subjects, in spite of the greater response of insulin secretion [Felig et al., 1974]. These observations emphasize that the liver is a major site of insulin action on glucose disposal.

(ii) Glucagon and catecholamines

Glucagon and catecholamines have been shown to exert antagonistic
effects on insulin actions on carbohydrate metabolism and hepatic glucose production. As mentioned in a review [Williams and Ensinck, 1966], catecholamines and glucagon:

(a) stimulate glycogenolysis in the liver, a process mediated by an accumulation of hepatic cyclic AMP. The latter agent activates a protein kinase, which in turn converts inactive glycogen phosphorylase to its active form, resulting in the glycogen breakdown.

(b) enhance gluconeogenesis from pyruvate by means of their stimulating effects on the formation of cyclic AMP. The latter agent increases the conversion of pyruvate to phosphoenol pyruvate [Exton and Park, 1969].

(c) increase proteolysis in muscle providing amino acids, and enhance lipolysis in adipose tissue supplying FFA and glycerol. As a result of these effects, there is an increase in the availability of gluconeogenic substrates to the liver [Williams and Ensinck, 1966].

Therefore, both hormones probably influence the carbohydrate homeostasis. An intravenous infusion of epinephrine in man can induce hyperglycemia without any changes in serum insulin level [Cerasi et al., 1971]. Conversely, epinephrine intravenously infused at low dose (0.3 μg/minute) for 2 hours augmented the insulin response following 25 g glucose administered intravenously, without altering the pattern of carbohydrate tolerance [Cardon et al., 1974]. A higher dose of epinephrine (0.6 μg/minute) infusion was observed to inhibit insulin secretion [Robertson and Porte, 1974]. It is likely that changes in insulin secretion induced by catecholamines vary according to the dosage of the
hormones. On the other hand, plasma total catecholamine levels rose, following an intravenous injection of 20 g glucose in normal man, to reach a peak at 3 minutes. This occurred 1 minute after the rise of plasma insulin [Robertson and Porte, 1974]. This phenomenon may have physiological importance in determining carbohydrate usage. However, the precise site of action of catecholamines remains to be determined.

The interactions of insulin and glucagon in terms of bihormonal modulation of glucose homeostasis by the liver have been recently discussed [Felig et al., 1976a]. It can be concluded from their review that in normal man, despite the fact that a rise in insulin and a fall in glucagon concentration in plasma were observed after ingestion of glucose [Felig et al., 1975], the primary factor determining glucose disposal was the insulin secretory response, rather than glucagon suppression. This conclusion is based on the work of Sherwin et al. [1976] who demonstrated that physiological hyperglucagonaemia (approximately 300 pg/ml, induced by continuous infusions of glucagon) had no effect on carbohydrate tolerance or insulin secretion, as compared with data recorded during a control saline infusion. Additionally, in response to a small increment in plasma glucose, splanchnic glucose production was reduced by 85%. The latter event is the function of increased insulin secretion (2-fold rise), since there was no change in arterial glucagon or peripheral glucose utilization [Felig and Wahren, 1971; Felig et al., 1974]. Felig et al. [1976a] also suggested that the primary physiologic function of glucagon is to prevent the hypoglycaemia induced by protein-stimulated insulin secretion, since a protein meal did not cause a fall in splanchnic glucose output in the presence of hyperinsulinaemia [Wahren et al., 1976]. This hypothesis was supported by the observation that an
infusion of glucagon to obtain the plasma level comparable to that
induced by a protein meal could abolish the insulin inhibiting effect on
the hepatic glucose output [Felig et al., 1976b].

On the other hand, infusion of glucagon (to attain plasma levels of
250-800 pg/ml) into insulin-independent diabetic subjects for 2 to 4
days did not change the blood glucose and ketone levels, as compared
with pre- or post-infusion periods [Sherwin et al., 1976]. Increased
blood glucose was noted in insulin-deficient juvenile-onset diabetics
infused with a similar dose of glucagon [Sherwin et al., 1976]. Thus,
the diabetogenic effects of glucagon (on physiological elevation) is
demonstrable only in the face of an absolute insulin lack [Felig et al.,
1976a].

The proposal of Felig and associates [1976a] that glucose disposal
is primarily determined by secretion of insulin was also confirmed by
the study of Schade and Eaton [1976]. In the latter study, there is a
lack of positive glycaemic response observed in the plasma during an
80 minute period of physiological elevation of plasma glucagon
(300 pg/ml) in normal subjects. In addition, when somatostatin (a hypo­
thalamic peptide) was infused in normal subjects to suppress the post­
absorptive concentration of both plasma insulin and glucagon for 6 hour
period [Lins and Efendić, 1976], there was a 10.6% decrease in plasma
glucose concentration during the first hour of infusion. Thereafter,
the plasma glucose level gradually increased until the end of 6 hours to
a slightly hyperglycaemic level, despite a continuing fall of both
plasma insulin and glucagon. Thus, hyperglucagonaemia is not required
for the development of hyperglycaemia [Lins and Efendić, 1976].

In contrast, Liljenquist et al. [1977] have suggested that basal
glucagon also plays an important role in regulating blood glucose
homeostasis in normal man. Selective reduction in arterial glucagon (by 50%) with basal insulin level maintained (induced by infusion of somatostatin and insulin) produces a marked fall in the net splanchnic glucose production. However, they also suggest the possibility of an increased sensitivity of liver to the basal amount of insulin during hypoglucagonaemia [Liljenquist et al., 1977].

(iii) Growth hormone and glucocorticoids

Both of these hormones have diabetogenic effects. Infusion of pharmacologic amounts of growth hormone stimulates lipolysis in man, as judged by an increased plasma FFA [Raben and Hollenberg, 1959]. The diabetogenic effect of growth hormone in man is probably a consequence of an induction of FFA release from adipose tissue [Hollobaugh et al., 1968], since an increased FFA concentration inhibits uptake of glucose and its metabolism by rat heart muscle in vitro [Randle et al., 1963]. However, physiologic amounts of growth hormone have no effect on lipolysis in vitro, neither under basal condition nor after stimulation with noradrenaline nor in the presence of hydrocortisone, as observed in culture of human adipose tissue [Nyberg and Smith, 1977]. On the other hand, growth hormone inhibits glucose uptake in muscle, as demonstrated in forearm muscle studies [Rabinowitz et al., 1965; Fineberg and Merimee, 1974]. Growth hormone also reduces the incorporation of glucose-1-14C into human adipose tissue triglycerides [Nyberg and Smith, 1977]. These effects of growth hormone lead to diminution of carbohydrate utilization.

Glucocorticoids exhibit antagonistic effects to insulin by several mechanisms, for example enhancement of lipolysis in rat adipose tissue [Jeanrenaud and Renold, 1960], inhibition of glucose transport into rat
muscle [Riddick et al., 1962] and rat fat cells [Czech and Fain, 1972], and promotion of hepatic gluconeogenesis, mediated in part by its amino acid releasing effect from muscle [Wise et al., 1973]. Glucocorticoids also antagonize the actions of insulin by stimulating the breakdown of muscle proteins, and by activating the key enzymes of gluconeogenesis in the liver [Williams and Ensinck, 1966]. Moreover, subjects placed on a prolonged glucocorticoid treatment have impaired carbohydrate tolerance [Conn and Fajans, 1956; Landau, 1965]. However, growth hormone and glucocorticoids appear to have only permissive effects on these metabolic activities. There is no definite evidence to show that these hormones play any significant roles in regulating glucose homeostasis in man during restriction of caloric intake [Cahill et al., 1970]. Moreover, a more recent study has shown that physiological levels of growth hormone fail to alter plasma FFA or glucose concentration in man in the presence of physiologic levels of insulin [Gerich et al., 1976].

One can therefore conclude that in humans, the liver is central to blood glucose homeostasis, and this reflects hepatic sensitivity to insulin effects. The latter hormone is a primary factor determining glucose disposal by the liver. The precise mechanism whereby insulin regulates carbohydrate metabolism in the liver as well as hepatic glucose intake and output are debatable. Insulin probably stimulates de novo synthesis of hepatic glucokinase [Borrebaek et al., 1970], resulting in the increases in glucose metabolism and glucose intake by the liver. Insulin also augments hepatic glycogen synthesis. Moreover, it inhibits hepatic glycogenolysis and gluconeogenesis as well as hepatic uptake of gluconeogenic substrates. The above findings cause, in part, a reduction of hepatic glucose production. Furthermore,
insulin presumably reduces the supply of glucose precursors. Its anti-
lipolytic effects suppress glycerol release from adipose tissue (see
sections 1.2 and 1.4); insulin also directly inhibits the mobilization
of amino acids from muscle [Pozefsky et al., 1969].

(b) Effects of Dietary Changes on Carbohydrate
Tolerance in Relation to Hormonal Regulation

(i) Starvation

The following metabolic events occur during brief fasting (3 to 4
days) in normal subjects Cahill et al., 1966; Cahill et al., 1970:

(a) glucose is produced mainly from hepatic gluconeogenesis,
whereas breakdown of hepatic glycogen contributes to only a
minor portion of hepatic glucose output.

(b) amino acids and glycerol, released from muscle and adipose
tissue respectively, serve as hepatic gluconeogenic substrates,
as well as pyruvate and lactate obtained from glycolysis in
muscle.

(c) FFA mobilized from adipose tissue are directly used for
metabolism of other tissues (muscle, heart, kidney, etc.), or
are taken up by the liver and partially oxidized to ketoacids
(acetoacetate and beta-hydroxybutyrate).

The latter substances serve also as the metabolic fuel of peripheral
tissues. When starvation is prolonged to 5 or 6 weeks, muscle protein
is depleted, gluconeogenesis decreases and the brain uses ketoacids for
a major fraction of its energy requirements [Cahill et al., 1970].

The provision and use of substrates in the body during caloric
depletion are controlled by several factors. Glucose-insulin feedback
mechanism was suggested to be a primary control for the release of peripheral fuel to provide energy metabolism during fasting [Cahill et al., 1966; Cahill et al., 1970]. A decrease in plasma glucose was associated with a 50% reduction in plasma insulin level, when normal subjects were completely fasted for 7 days [Cahill et al., 1966]. On the other hand, a phenomenon called "starvation diabetes", characterized by a decrease in carbohydrate utilization, was previously observed in man subjected to starvation [Goldblatt, 1925]. This observation was regularly confirmed in several studies, in which impairment of carbohydrate tolerance (either an oral or intravenous administration) was found after a 3- or 7-day fast in normal persons [Unger et al., 1963; Cahill et al., 1966; Fink et al., 1974]. A delayed rise with prolonged hyperinsulinaemia following an oral GTT [Unger et al., 1963] or prompt response with a low peak of insulin secretion after an intravenous injection of glucose [Cahill et al., 1966] was reported in starved man. Moreover, a 48-hour starvation in healthy volunteers caused decreases in the glucose disposal rate as well as in the total, initial and late phases of insulin secretion. These data were recorded following glucose challenge by a priming injection prior to a continuous intravenous infusion [Fink et al., 1974]. A deterioration of glucose tolerance after 1 or 2 weeks of fasting was also observed in obesity without associated diabetes mellitus [Genuth 1966; Sussman, 1966]. The hyperinsulin response to glucose found in obese subjects is unaltered during a period of starvation [Genuth 1966; Sussman, 1966]. Moreover, a 14-day fast in overweight subjects, followed by refeeding with a basic diet to stop ketosis, results in an impairment of oral glucose tolerance and a delayed rise in insulin secretion [Tzagournis and Skillman, 1970]. A low insulin/glucose ratio was noted at the
It is well established that caloric deprivation in humans leads to diminished glucose utilization. However, mechanism(s) responsible for this starvation type of glucose intolerance remain obscure. Some metabolic changes in the pancreatic beta cells may be considered in causation: a reduction in insulin release, probably reflecting a decreased response of the beta cells to glucose stimuli; or an inability of the beta cells to secrete insulin promptly in response to acute glucose load as found in the early state of diabetes mellitus.

Consideration has been given to the roles played by other substances, mainly on the alteration in hormones released from the pituitary, adrenal cortex and alpha cells of the pancreas, during fasting. Growth hormone, cortisol and glucagon have been shown to act antagonistically to the effects of insulin, as has already been described. Moreover, growth hormone secretion in man is stimulated by hypoglycaemia [Roth et al., 1963a; Luft et al., 1966]; and the blood level of growth hormone is raised during fasting [Roth et al., 1963b; Marks et al., 1965; Merimee et al., 1976]. However, there are fluctuations in the growth hormone concentration after caloric restriction in adults, as judged by the values at base-line or following an intravenous glucose tolerance [Cahill et al., 1966]. On the other hand, the basal growth hormone levels in fasted obese subjects (a 14-day fast) are relatively lower than those recorded prior to fasting [Tzagournis and Skillman, 1970]. Therefore, there is no definite information to support that the raised growth hormone levels (if they occur) during starvation in humans take part in causing subsequent intolerance to glucose. There are no changes in cortisol concentrations in the plasma [Tzagournis and Skillman, 1970] or in the urine [Cahill et
al., 1966; Tzagournis and Skillman, 1970], during prolonged fasting. Thus, the impairment of carbohydrate utilization developed in fasting man is not due to increased secretion of glucocorticoids.

Hyperglucagonaemia was found after a brief starvation in man [Unger et al., 1963; Cahill et al., 1970; Stout et al., 1976]. Glucagon has stimulating effects on hepatic gluconeogenesis and glycogenolysis. This hormone may, therefore, be responsible for the development of starvation diabetes [Unger et al., 1963]. Furthermore, Cahill et al. [1970] have pointed out that glucagon possibly plays an important role in the regulation of amino acid metabolism by the liver during a period of starvation. Their conclusion was made in part from studies of Felig et al. [1969] who showed that plasma glucagon levels observed in the post-absorptive state and after starvation, correlated with the fractional hepatic extraction of alanine. Cahill et al. [1970] also used evidence derived from a report of Marliss et al. [1970] that an infusion of small amounts of glucagon into fasting man resulted in a significant reduction of amino acid concentration, indicating a sensitivity of amino acid metabolism to the effect of glucagon. A more recent review by Felig et al. [1976a] has suggested that the rise in glucagon level during a 1 to 7 day period of starvation has no significant effect on hepatic gluconeogenesis and glycogenolysis, but the fall in insulin concentration is responsible for the stimulation of two metabolic parameters. Their proposal is based on data provided by previous work of Felig et al. [1976b], who showed that the stimulatory action of physiologic amounts of glucagon, infused intravenously to normal subjects, on splanchnic glucose production was transient. An increased splanchnic glucose output was observed 7-15 minutes after glucagon infusion, then it returned to basal level within 22-30 minutes, despite
the continuation of hyperglucagonaemia, and stable concentrations of plasma glucose and insulin [Felig et al., 1976b].

An increase in FFA concentration in the circulation during fasting in normal subjects [Unger et al., 1963; Cahill et al., 1966; Brodows et al., 1976; Stout et al., 1976] or in obesity [Tzagournis and Skillman, 1970; Marliss et al., 1970; Wildenhoff et al., 1975] has been suggested as a cause of carbohydrate intolerance and insulin insensitivity. In studies with isolated rat heart muscle, Randle et al., [1963] has proposed that elevated plasma FFA, due to an increased lipolysis of triglycerides in adipose tissue and muscle, account for impaired glucose metabolism in muscle. The latter phenomenon leads to insulin insensitivity and frequently glucose intolerance; and the sequence of events was called the "glucose-fatty acid cycle" [Randle et al., 1963]. At present, there is no evidence in humans to support that high concentrations of plasma FFA can induce insulin resistance [Tzagournis and Skillman, 1970], or can stimulate insulin secretion [Carroll and Nestle, 1972], or can alter the rate of glucose disposal [Ruderman et al., 1969]. The raised FFA level during starvation may be one of the less important factors causing carbohydrate intolerance.

In brief, a starvation type of carbohydrate tolerance curve may be the result of metabolic adaptations of various tissues in order to preserve glucose for brain metabolism during a lack of exogenous energy supply. Changes in plasma concentrations of several hormones and metabolic substrates are the physiological response of the body to restricted caloric intake. Insulin concentration in plasma appears to be a primary determinant in regulating the supply of peripheral fuel. The cause of impaired carbohydrate utilization has been proposed: there is a reduction in insulin secretion due to a diminished store of insulin
in the beta cells or a defect in the secretory response of the beta cells to stimuli. On the other hand, metabolic changes in the liver and peripheral tissues are possibly involved in deterioration of the glucose tolerance during caloric depletion in humans. Studies concerning these aspects will be discussed in the context of carbohydrate deprivation.

(ii) Carbohydrate deprivation

An impairment of carbohydrate tolerance is not only a consequence of caloric restriction but is also observed after isocaloric low carbohydrate feeding in normal subjects. The latter dietary manipulation results in a deterioration of oral GTT with a delayed rise and prolonged hyperinsulinaemia [Hales and Randle, 1963a; Wales et al., 1967; Permutt et al., 1976], or a slight impairment of the intravenous GTT with a significantly diminished peak of insulin secretory response [Danforth, 1971]. The effects of a low carbohydrate diet on basal levels of plasma glucose or insulin are controversial. Normal weight subjects taking this type of diet had low basal concentrations of plasma glucose and insulin [Danforth, 1971], or had only low basal glucose level without alteration in insulin concentration [Permutt et al., 1976]. In one report, hyperglycaemia with hyperinsulinaemia in the postabsorptive state was observed in subjects consuming a diet containing less than 50 g of carbohydrate [Hales and Randle, 1963a]. The reason for these divergent results cannot be given. On the other hand, the cause of impaired carbohydrate disposal during carbohydrate deprivation is not fully understood. A decrease in sensitivity of tissues to insulin effects is excluded, since subjects have a normal hypoglycaemia response to exogenous insulin administration [Danforth, 1971]. Insulin antagonism is unlikely to
occur during feeding an isocaloric low carbohydrate diet, since there is no significant alteration in the plasma concentration of growth hormone in the basal state [Danforth, 1971; Permutt et al., 1976] or following an oral GTT [Permutt et al., 1976]. Basal plasma glucagon level was unaltered but an impaired suppression of glucagon by glucose ingestion was observed in normal subjects fed a carbohydrate-deficient diet. However, the physiological significance of this phenomenon in relation to the impaired glucose tolerance is unknown [Permutt et al., 1976]. An impaired insulin secretory response of the pancreas to glucose loads during this dietary manipulation is more likely to be a contributor to the impairment of carbohydrate tolerance. This is because carbohydrate depletion in normal subjects produces a significant decrease in the early insulin release (first 2 hours of oral GTT) followed by an increase in the late insulin release (between 3-4 hours of oral GTT), without changes in the total insulin secretion, as compared with the control period [Permutt et al., 1976].

The amount of carbohydrate in the diet appears to be a more critical factor in modifying the insulin secretory pattern than low caloric intake. Studies in obesity [Grey and Kipnis, 1971] have demonstrated that isocaloric and hypocaloric diets, both containing high percentages of carbohydrate (53% and 72% respectively), produce increases in fasting plasma insulin level and plasma insulin response to an oral GTT. Carbohydrate restriction on both isocaloric and hypocaloric diet has the converse effect [Grey and Kipnis, 1971].

On the other hand, evidence has been presented supporting the concept that an isocaloric substitution of fat for dietary carbohydrate is, in part, responsible for glucose intolerance [Anderson et al., 1973]. Fat feeding in man results in an impaired carbohydrate tolerance
Moreover, normal subjects taking an isocaloric diet containing low carbohydrate with a normal quantity of fat [Anderson and Herman, 1972], or having a hypocaloric diet of similar composition [Wilkerson et al., 1960] can maintain normal carbohydrate tolerance. In addition, Anderson et al. [1968, 1973] found decreases in fasting plasma insulin and a delayed maximum rise of insulin response to an oral GTT. The actual mechanism by which an excessive amount of fat in the diet produces a deterioration of carbohydrate tolerance is speculative. Peripheral sensitivity to insulin may be reduced by a high fat-low carbohydrate diet, as shown by an elevation of basal plasma FFA and a delayed suppression of plasma FFA by an oral glucose load [Hales and Randle, 1963a], reflecting an increased lipolysis or a decreased esterification in adipose tissue.

Therefore, a diet with low carbohydrate-high fat content can lower glucose tolerance, possibly by a decreased response of the pancreas to stimuli. On the other hand, abnormal glucose tolerance could have been related to the mechanism whereby liver regulates glucose homeostasis. In this regard, activity of human hepatic glucokinase, the rate-limiting enzyme of the phosphorylation of glucose, falls after taking a low carbohydrate-high fat diet [Borrebaek et al., 1970]. There is no evidence to show that in humans the latter diet will enhance the rate of hepatic gluconeogenesis. Data obtained from experimental animals have shown an enhancement of hepatic gluconeogenesis from alanine and pyruvate in vitro [Eisenstein et al., 1974].

Recently, it has been established that the major cause of an impairment of glucose tolerance following a hypocaloric low carbohydrate-
high fat diet is a delay in the hepatic uptake of glucose load [Jackson et al., 1973b]. In this work, normal volunteers maintained on the latter dietary regimen for 7 days showed no changes in the rate of forearm glucose uptake, or growth hormone secretion, following an oral glucose administration. A slightly delayed rise in serum insulin response with subsequent hyperinsulinaemia was observed in these subjects but this change did not correlate with the markedly impaired glucose tolerance. Thus they have suggested that the delayed hepatic uptake of glucose following an oral GTT during carbohydrate deprivation is primarily the consequence of a low hepatic glucokinase activity [Jackson et al., 1973b].

(iii) Isocaloric high carbohydrate feeding

Feeding an isocaloric high carbohydrate diet to normal subjects for one week results in an improvement of oral glucose tolerance and enhancement of insulin sensitivity to exogenous insulin [Himsworth, 1935]. These findings are in accord with later studies which show that normal individuals maintained on a carbohydrate-rich diet have an increased utilization of glucose without significant changes in both pattern and amount of insulin secretion. This observation reflects an improved sensitivity of tissues to the effects of endogenous insulin [Anderson et al., 1968; Anderson et al., 1973; Brunzell et al., 1971]. Apart from an improvement of insulin sensitivity, a high carbohydrate diet stimulates both early insulin release from a small readily available pool of the beta cells, and the glucose disappearance rate (Kg), in both normal and mildly diabetic subjects. These changes were measured after an intravenous injection of 20 g glucose, and were compared with the data obtained during taking control diets [Lerner et al., 1971].
Normal men eating an isocaloric or hypercaloric high carbohydrate diet (containing 80% of calories as carbohydrate) for 8-10 days, had a significant decrease in serum growth hormone level which was estimated as the sum of all hourly samples in 24 hours [Merimee et al., 1976]. In the same study, growth hormone secretion/24 hours in these men on an isocaloric diet of high fat or high protein or on a hypercaloric diet of basic composition did not differ from data recorded during the control periods [Merimee et al., 1976]. Even though the actual role of this hormone on carbohydrate utilization is unknown, it is conceivable that from the above findings an insulin antagonist, growth hormone, in the plasma is reduced by high carbohydrate content in the diet.

The effects of isocaloric high carbohydrate diets on carbohydrate tolerance and insulin secretion in the obese individuals seem to be different from that in normal subjects. This can be seen in studies of Sims et al. [1973] in normal subjects and after an induction of experimental obesity. They found that an increased dietary carbohydrate/fat ratio was associated with an improved oral GTT only in subjects with normal weight, and was without effect on carbohydrate tolerance after weight gain. There were no changes in plasma insulin response following glucose loads [Sims et al., 1973]. In their studies, insulin resistance was demonstrable in subjects after an increase in weight, i.e., an elevation of approximately 50% in basal insulin secretion, and impairment of carbohydrate utilization [Sims et al., 1973]. It is possible that obesity as such, or factors associated with obese state, can modify the effect of a high carbohydrate diet.

An isocaloric high carbohydrate diet in normal subjects can restore the low activity of hepatic glucokinase (induced by a carbohydrate-free diet) to normal level [Borrebaek et al., 1970], resulting in enhancement
of glucose disposal by the liver.

(iv) Overfeeding

An excess caloric consumption leads to an increase in fat deposition. Any metabolic changes observed in obesity may be secondary to overeating or obesity itself. Olefsky et al. [1975] have studied the effects of overfeeding with a diet of standard composition in normal weight subjects. They have reported that the hypercaloric intake produces increases in fasting glucose and insulin concentrations, at the end of the first week, before significant gain in weight has occurred. In addition, the insulin secretory response to an oral GTT is increased, but the blood glucose following the administered glucose load is not altered [Olefsky et al., 1975]. These findings are similar to the work of Nestel et al. [1970], who showed that overfeeding with a high carbohydrate diet to normal subjects for 7 days led to a 2-fold increase in the insulin secretion following an intravenous GTT. However, hyperinsulinaemia induced by the overfeeding is not associated with a state of insulin resistance. This is shown by the steady-state plasma glucose levels during a course of insulin tolerance test are comparable both before and after overfeeding [Olefsky et al., 1975].

In conclusion, the liver is a major site for glucose disposal and is central for maintaining glucose homeostasis in normal man. Insulin is the primary factor determining these metabolic events. Nutritional factors such as caloric intake and diet composition also influence carbohydrate disposal by the liver. A caloric restriction as well as a low carbohydrate-high fat feeding cause an impaired carbohydrate tolerance, possibly mediated by a reduction in insulin secretion or a
decreased response of the pancreas to glucose stimulus or a low activity of hepatic glucokinase. An isocaloric high carbohydrate diet results in an improvement of carbohydrate utilization and increases in insulin sensitivity and activity of hepatic glucokinase. Overfeeding with a basic diet leads to increases in basal insulin concentration and insulin secretory response to glucose load without any change in carbohydrate tolerance.
2.1 SUBJECTS AND DIETARY MANIPULATIONS

Subjects were normal weight student volunteers and overweight patients. They were admitted in a metabolic ward. Informed consent was obtained from all subjects after a full explanation of the procedures involved. Each subject was given a physical examination before the study. Relevant clinical data are given in Chapter 3 (Studies of Adipose Tissue Metabolism) and in Chapter 4 (Studies of Carbohydrate Tolerance). Ideal body weights were calculated from tables of standard body weights [Society of Actuaries, 1959]. None of the subjects received any medication for at least 3 weeks before or at any time during the studies. Their body weights were recorded daily before breakfast. Subjects were allowed to maintain their normal activities.

All subjects were placed on an isocaloric standard diet providing, on average, 15% of total calories from protein, 39% from fat and 46% from carbohydrate (P:F:C = 15:39:46%) for about 1 week. They were then maintained on one of the experimental diets for 1 week or 2 weeks: isocaloric high carbohydrate diet (average of P:F:C = 13:13:74%), hypercaloric high carbohydrate diet (average of P:F:C = 11:9:80%), hypercaloric high fat diet (average of P:F:C = 11:57:32%) and caloric restricted diet (average of P:F:C = 22:27:51%). The composition of the diets was calculated from standard tables [Thomas and Corden, 1970].
Meals were consumed at 8.00 a.m., 12.30 p.m. and 5.30 p.m. A standard high carbohydrate breakfast containing 750 calories (P:F:C = 8:25:67%) was fed to each subject throughout the entire study except those who were undertaking a caloric deprivation regimen. The total carbohydrate content of this meal was 120 g. The percentages of total calories in the breakfast derived from starch, sucrose, fructose and lactose were 31%, 21%, 11% and 4%, respectively. The composition and quantity of calories given at lunch and dinner were adjusted to give the required daily intake of calories, protein, fat and carbohydrate. Specific details of caloric intake and dietary composition of individuals are shown in Appendices 1 and 2.

In subjects participating in the adipose tissue study (Chapter 3), 5 blood samples per week were taken following an overnight fast. Adipose tissue biopsies were performed 2 to 3 times per week, 1½ hours after the standard high carbohydrate breakfast, and during control and experimental diet feeding. In order to study the effects of starvation on adipose tissue metabolism, the biopsies were taken after an overnight fast in both control and fasting periods.

In subjects in which the effects of dietary changes on carbohydrate tolerance were examined (Chapter 4), blood samples were withdrawn twice a week, both before and at half-hourly intervals (for 2 hours) after the standard high carbohydrate meal, in both control and overfeeding periods.

2.2 ADIPOSE TISSUE BIOPSY

Adipose tissue biopsies were performed by Dr. R.B. Goldrick, who used a needle aspiration technique described by Diengott and Kerpel [1967]. Samples of fat (100-600 mg) were obtained from subcutaneous
tissue from the gluteal region of subjects under local anaesthesia (2% xylocaine). When used in lean subjects, the biopsy needle was equipped with a modified outer shaft in which the length of the cutting slot was reduced to 2.5 cm. With this modification, biopsies were obtained after introducing the needle 4 cm parallel to the skin surface. Subjects were kept in bed for one hour before the biopsies were performed and smoking was prohibited.

Local anaesthesia used prior to biopsies of subcutaneous fat may influence the metabolic activity of adipose cells. It has been demonstrated in study with isolated rat fat cells that local anaesthetics inhibit the lipolytic effects of several lipolytic agents and reduce the stimulatory action of insulin on glucose uptake [Hales, 1970]. However, studies with isolated human fat cells have shown that the cells obtained from donors under spinal anaesthesia or general anaesthesia have a comparable rate of lipolysis observed during both basal incubation and the addition of catecholamines [Galton and Bray, 1967; James et al., 1971]. In the present study, any effect of the local anaesthetic, used during the biopsy procedure, on adipose tissue metabolism should be a systematic error, since subjects themselves were their own controls.

2.3 PREPARATION OF ADIPOSE TISSUE SAMPLES

The sample of adipose tissue fragments obtained from needle biopsy was immediately transferred to the laboratory in isotonic saline at 37 °C. The specimen was processed within 5 minutes after removal from the donor. Any contaminating blood and fibrous connective tissue were dissected away with scissors. Adipose tissue shreds were cut into small pieces (approximately 5 mm in dimension) and gently blotted. Then they
were transferred to a preweighed flask containing 20 ml of a buffer. The whole flask was weighed again to assess the wet weight of the added tissues, and was preincubated at 37 °C for 30 minutes. The weight of tissue was recorded for selection of an appropriate protocol used for the subsequent definitive incubation (2 hours).

2.4 GENERAL CONDITIONS OF INCUBATION

Incubation of adipose tissue pieces was performed in buffer in a siliconized flask [Goldrick, 1967a] at 37 °C, under an atmosphere of air. The flask was capped with a rubber serum stopper and incubated in a shaker water bath set at 60 cycles/minute. The buffer was Krebs-Ringer phosphate (KRP), pH 7.4, containing half the suggested concentration of calcium ions [Umbreit et al., 1964]. The composition of the buffer was: NaCl (119.4 mM), KCl (4.8 mM), CaCl$_2$ (1.3 mM), MgSO$_4$.7H$_2$O (1.2 mM), and phosphate buffer (16.3 mM). It was freshly prepared for each experiment and contained 1 mg glucose and 40 mg fraction V bovine albumin per ml. The glucose served as a metabolic fuel [Ho et al., 1970] and albumin was an acceptor for FFA released from adipose tissue [Vaughan, 1962]. Final pH of the phosphate buffer containing albumin and glucose was adjusted to 7.4 by adding a few drops of 1.0 N NaOH.

2.5 DETERMINATIONS OF ADIPOSE CELL SIZE AND NUMBER

Adipose cell diameter was measured by a photomicroscopic method [McLeod et al., 1972] on suspensions of isolated fat cells. The latter cells were prepared from small subsamples of tissue pieces (25-50 mg) incubated with 10 mg of collagenase/ml phosphate buffer in a siliconized
tube. Incubation was performed at 37 °C for 1½-2 hours. A small aliquot of the cell suspension was placed on a siliconized glass slide, and covered by a siliconized cover glass supported by a thick layer of vacuum grease. The free cells were photographed in planes of maximal diameters using a Zeiss photomicroscopic system [Carl Zeiss, Oberkochen/Wuertt, West Germany] at a magnification of X 85.5 mm. The negative photomicrographs were used to measure cell diameters. At least 500 fat cells were semiautomatically measured with a Zeiss particle size analyzer TCZ 3 (obtainable from Carl Zeiss, Oberkochen/Wuertt, West Germany), to obtain a normal frequency distribution curve of the cell diameters.

Mean fat cell volume was calculated from the mean and variance of the diameters using the formula of Goldrick [1967b]. Fat cell weight (µg of triolein/cell) was determined from the product of the mean cell volume and the density of human fat triglycerides (0.915 g/ml [Keys and Brozek, 1953]), as suggested by Hirsch and Gallian [1968]. The total lipid content of adipose tissue is almost equivalent to its mass of triglycerides [Hirsch et al., 1960]. Moreover, adipose cell lipid calculated from direct measurement of cell diameter is comparable with that obtained from the total lipid content of the tissue divided by the number of fat cells [Hirsch and Gallian, 1968]. Therefore, the number of micrograms of triolein per cell was assumed to represent fat cell size in this study.

The total number of fat cells in a given sample of adipose tissue was calculated by dividing total triglyceride content of the tissue by mean weight of lipid per fat cell [Hirsch and Gallian, 1968]. The total lipid content (mg of triolein) of adipose tissue was measured by the carboxyl ester method [Skidmore and Entenman, 1962]. In the present
work, all metabolic parameters measured in adipose tissue studies were expressed as metabolic activity per $10^6$ adipose cells.

2.6 ESTIMATION OF LIPOLYSIS AND ESTERIFICATION IN ADIPOSE TISSUE

This was determined by a modification of the balance method of Vaughan [1962]. Simultaneous estimation of lipolysis and esterification in human adipose tissue was achieved by measuring both release of glycerol into the incubation medium, and total FFA production in the incubation system (net change in the concentrations of FFA in tissue plus medium). The quantity of glycerol release was taken as an index of lipolysis, since glycerol was metabolized only to a small extent in human adipose tissue [Koschinsky and Gries, 1971]. It is theoretically assumed that 1 mole of glycerol produced represents the production of 3 moles of FFA. In this study, glycerol production has been expressed as its equivalent in FFA concentration (moles of glycerol $\times$ 3) and used as a rate of lipolysis. Therefore, esterification rate in adipose tissue can be obtained according to the formula: esterification of FFA = (glycerol release $\times$ 3) - net change in FFA in the incubation system (net FFA production or utilization). All metabolic parameters were expressed as $\mu$ mole FFA/$10^6$ adipose cells/2 hours.

After preincubation, the tissues were blotted and approximately one fifth of the sample was used for measurements of tissue FFA and triglyceride concentration. The concentration of FFA/unit weight of triglycerides was used to calculate the tissue FFA content at the beginning of the 2 hour incubation period, hereafter referred to as the zero time tissue FFA ($T_0$ FFA). The remaining pieces of tissue were incubated in fresh KRP buffer (100 mg tissue/ml) for 2 hours. Fresh
buffer was also incubated in a separate flask for 2 hours to serve as a control \((M_0)\) for changes in the glycerol and FFA concentrations of the medium containing the sample of adipose tissue. After 2 hours of incubation, adipose tissue pieces were removed from the medium. The latter was used to measure concentration of glycerol and FFA \((M_{2hr})\).

The concentration of FFA/unit weight of triglycerides in the incubated tissue was used to derive the 2 hour tissue FFA \((T_{2hr} FFA)\). Net change in FFA was calculated from the change in FFA content of the tissues \((T_{2hr} - T_0)\) plus the change in FFA concentration in the incubation medium/unit wet weight of triglyceride \((FFA \text{ release or uptake} = M_{2hr} - M_0)\). Net glycerol production was calculated from the change in glycerol content of the incubation medium \((M_{2hr} - M_0)\)/unit weight of triglyceride. Esterification was calculated by subtracting the net change in FFA from the theoretical production of FFA calculated from the release of glycerol.

The changes in FFA concentration of the tissue \((T_{2hr} - T_0)\) and in the incubation medium \((M_{2hr} - M_0)\) have been listed separately in the results of Chapter 3 (Section 3.3). These parameters proved to be independent of each other and showed different responses to dietary manipulations.

### 2.7 PRELIMINARY STUDY USING THE BALANCE METHOD OF VAUGHAN

Simultaneous estimation of lipolysis and esterification in rat adipose tissue in vitro by a nonisotopic balance method was developed by Vaughan [1962]. The assumption on which this method for measuring triglyceride breakdown and synthesis in rats were based, have been reviewed [Vaughan, 1962; Steinberg and Vaughan, 1965; see Section
2.13). The balance method has been applied for use in the studies with human adipose tissue in vitro [Kjellberg and Östman, 1971; Lisch et al., 1973].

A preliminary study on lipolysis and esterification was also performed by Dr. R.B. Goldrick. Adipose tissue specimens were obtained from subjects at elective surgery under general anaesthesia. The preparation of adipose tissue and incubation conditions as well as estimations of FFA and glycerol concentrations were the same as in the present study. In these experiments, there was no preincubation of the tissue, and tissue glycerol concentration was measured to obtain the net production of glycerol in the incubation system. This was accomplished by measuring tissue concentrations of glycerol in homogenates of adipose tissue before and after different lengths of incubation. FFA levels in adipose tissue were also estimated in extracts of the same homogenates. Metabolic parameters were expressed as activity/g of adipose tissue.

Some of the relevant results are shown in figure 2.1. Incubation for ½ hour reduced concentrations of the preformed tissue glycerol to very low levels which were stable throughout the 2 hour incubation (Fig. 2.1a). Tissue FFA concentration was also substantially reduced during ½ hour incubation; however, further significant reduction in tissue FFA almost invariably occurred during incubation (Fig. 2.1a). The study also showed that the rates of lipolysis, esterification, and glycerol release into the incubation medium were linear (Fig. 2.1b and 2.1c respectively).

In the present investigation, preincubation of adipose tissue was performed for ½ hour. Therefore, analysis of tissue glycerol concentration was not required to calculate the net production of glycerol. The change in FFA concentration of the tissues was also taken
Figure 2.1: (a) Changes in concentration of adipose tissue FFA (○—○) and glycerol (●—●) during incubation in vitro for 2½ hours.

(b) Relationship between lipolysis (○—○) or esterification (●—●) and incubation times.

(c) Time course of the release of glycerol (●—●).

Metabolic parameters were expressed as μmoles/g of adipose tissue (AT).

Human subcutaneous adipose tissue taken during surgery was incubated for 2½ hours (without preincubation) in KRP buffer containing glucose 1 mg/ml and bovine albumin 40 mg/ml. Tissue FFA and glycerol concentrations were determined in tissue homogenates. The rate of lipolysis and esterification were estimated by the balance method of Vaughan. Each point represents the mean of duplicate incubations.
into account in calculation of total FFA production or utilization in the incubation system.

2.8 MEASUREMENT OF GLYCEROL CONCENTRATION IN THE INCUBATION MEDIUM AND PLASMA

For the measurement of glycerol, aliquots (in duplicate) of incubation medium or heparinized plasma were deproteinized with perchloric acid (15% w/v), neutralized with saturated potassium bicarbonate and assayed enzymatically [Garland and Randle, 1962]. The assay mixture contained the following: phosphoenol pyruvate 16.25 mg, ATP 62.5 mg, NADH 3.547 mg, lactate dehydrogenase 0.25 ml (5 mg/ml), pyruvate kinase 0.25 ml (10 mg/ml) in 25 ml triethanolamine, buffer pH 7.7. The latter buffer contained triethanolamine hydrochloride (154.1 mM), KCl (3.09 mM) and MgSO₄·7H₂O (9.36 mM). The reaction system comprised 0.5 ml of neutralized perchloric acid extract (protein-free supernate) of the sample, 0.5 ml of the assay mixture and 20 µl of glycerokinase (5 mg/ml). The assay mixture was adapted for fluorimetry by reducing the concentration of NADH (MW 709.4) to 100 nmoles in a final volume of 1 ml. The reactions of the assay are as follows:

\[
\begin{align*}
\text{(a)} \quad & \text{glycerol} + \text{ATP} \rightarrow \text{alpha-glycerophosphate} + \text{ADP} \\
\text{(b)} \quad & \text{ADP} + \text{phosphoenol pyruvate} \rightarrow \text{pyruvate} + \text{ATP} \\
\text{(c)} \quad & \text{pyruvate} + \text{NADH} \rightarrow \text{lactate} + \text{NAD}
\end{align*}
\]

The conversion of NADH to NAD by lactate dehydrogenase was measured in a spectrophotofluorometer (Aminco-Bowman, American Instrument Company, Inc., Maryland, U.S.A.), at excitation and emission wavelengths of 340
and 460 nanometers respectively. A change in emission was read from a scale expansion accessory at 1/4 minutes after the addition of glycerokinase. Internal standards and appropriate reagent blanks were included in each assay, which measured glycerol over the range of 5-60 nmoles/ml reaction mixture.

2.9 MEASUREMENT OF FFA CONCENTRATION IN ADIPOSE TISSUE, THE INCUBATION MEDIUM AND PLASMA

Lipids from adipose tissue or FFA from incubation medium or plasma were recovered by the single extraction technique of Dole and Meinertz (1960). In the case of adipose tissue pieces, the samples were added in Dole's extraction mixture, in the proportion of 100 mg of wet weight per 5 ml solvent (isopropanol:heptane:1.0 N H₂SO₄ = 4:1:0.1 [v/v/v]). Aliquots of incubation medium (0.3 ml) or plasma (0.5 ml) were extracted in 5 ml of the solvent. The extraction tubes were shaken intermittently for 48 hours at 4 °C to ensure complete recovery of triglycerides and FFA. The solvent then was converted to a two system by the addition of water and heptane (3 ml of each to 5 ml solvent). Aliquots of the upper phase of the lipid extract were evaporated to dryness at 60 °C under a stream of air, and lipids were redissolved in chloroform. FFA concentrations were measured as complexes with ⁶³NiCl₂ [Goldrick et al., 1972b; Reardon et al., 1973] by a method modified from a radiochemical assay of long-chain fatty acids according to Ho [1970]. One ml of the chloroform solution was pipetted into a Pasteur pipette, so modified that the narrow end was about 0.5 inch long and sealed at the tip. Then 0.1 ml of nickel chloride reagent which contained 1 volume of 1.0 nCi Nickel-63 (352 μg ⁶³NiCl₂.6H₂O, S.A. 12.06 mCi/mg Ni) plus 15 volumes of 1.0 M triethanol-amine buffer, was added into the same tube. The estimation was
performed in triplicate. The mixture was vigorously mixed and then centrifuged. The tip of the pipettes was broken off, and the lower chloroform phase containing nickel soap of FFA was transferred into a counting vial. The chloroform was evaporated to dryness and the $^{63}$Ni-FFA complexes were dissolved in 15 ml of toluene scintillation fluid (4 g of 2,5-diphenyloxazole and 50 mg of 1,4-bis[2-(4-methyl-5-phenyl-oxazole)]-benzene/L). The radioactivity was counted in a Packard Tricarb Liquid Scintillation Spectrometer (Model 3003). Palmitic acid was used as the internal standard. Under these conditions there was a linear log:log relationship between the observed counts per minute and the concentrations of FFA over the range 0.001-2.0 μmoles/ml of standard solution. The estimation of FFA was not affected by the presence of triglycerides.

2.10 BLOOD ANALYSES

Blood samples were collected, without venous occlusion, in chilled heparinized tubes. The plasma was separated at 4 °C within 1 hour and stored at -20 °C. Samples of plasma from each subject were analyzed in a single batch at the end of the study to avoid inter-assay variation and artifacts caused by repeating thawing and refreezing of the samples. Plasma insulin was determined in triplicate with the double antibody radioimmunoassay method of Hales and Randle [1963b] using $^{125}$I-Insulin assay kit. Plasma FFA were measured by a radiochemical assay utilizing $^{63}$NiCl$_2$ [Goldrick et al., 1972b]. Plasma glycerol was enzymatically assayed [Garland and Randle, 1962]. Plasma cholesterol and triglycerides were analyzed on an autoanalyzer [Köhring and Kattermann, 1974]. Plasma glucose was estimated enzymatically [Huggett and Nixon, 1957].
2.11 RADIOACTIVE AND CHEMICAL REAGENTS

$^{63}$Ni (Nickel chloride in 0.1 M HCl, Code No. NBS 1) used in the measurement of FFA was supplied from the Radiochemical Centre Ltd., Amersham, England.

Insulin radioimmunoassay kit (Insik-1) was derived from CAE-IRE-SORIN Association, Saluggia, Italy.

Bovine albumin powder (fraction V from bovine plasma) containing 5 μmoles FFA/g, used in the studies of adipose tissue metabolism in vitro was purchased from Armour Pharmaceutical Company Ltd., Eastbourne, England.

Collagenase used for preparation of isolated fat cells was obtained from Worthington Biochemical Corporation, New Jersey, U.S.A.

Tripalmitin and palmitic acid standards used in the measurements of lipid content of adipose tissue and FFA respectively, were obtained from Serdary Research Laboratories, London, Ontario, Canada.

Enzymes and biochemicals used for the assay of glycerol were supplied from Boehringer, Mannheim GmbH, Germany. These were lactate dehydrogenase, glycerokinase, pyruvate kinase, adenosine-5'-triphosphate (ATP), reduced nicotinamide-adenine dinucleotide (NADH), and phosphoenol pyruvate. Only the glycerol standard was obtained from David G. Bull Laboratory Pty. Ltd., Canterbury, Victoria.

All other chemicals employed were analytical grade (A.R.).

2.12 STATISTICAL ANALYSES

Comparisons were made by the analysis of variance [Bailey, 1969] using paired data from each subject. When significant interactions
occurred between primary sources of variation such as subjects and diets, the mean square of the appropriate interaction was employed as the divisor in computing the variance ratio. Correlation coefficients were computed by covariance analysis [Bailey, 1969] after inspection of the scatter diagrams showed an even distribution of the data. In both analyses, p values of 0.05 or less were considered to be significant.

2.13 DISCUSSION ON METHODOLOGY AND SOME ASPECTS OF EXPERIMENTAL DESIGN

The nonisotopic balance method of Vaughan [1962] was applied to measure the rates of lipolysis and esterification in human adipose tissue in vitro in this study for the following reasons.

(a) Both lipolysis and esterification can be simultaneously measured.

(b) Not only lipolysis and esterification but also other parameters of adipose tissue metabolism (tissue FFA concentration, FFA release) can be used for studying the metabolic patterns in adipose tissue.

(c) The nonisotopic balance method is an appropriate and reliable method for assessment of the rates of lipolysis and esterification in adipose tissue (as suggested by Kjellberg and Östman, 1971 and by Lisch et al., 1973), because the rate of lipolysis is estimated as the production of glycerol (which was considered as a more accurate index than the release of FFA [Björntorp and Östman, 1971]). Another reason is to avoid the use of radioactive substrates (glucose-\(^{14}\)C or palmitate-\(^{14}\)C) for the measurement of the rate of esterification, since the dilution of isotopic substrates by metabolites produced by adipose
tissue may give incorrect information for the rate of triglyceride synthesis [Kjellberg and Östman, 1971; Lisch et al., 1973].

The balance method was developed by Vaughan [1962] for measuring the rate of triglyceride breakdown and synthesis in rat epididymal fat pads in vitro. The validity of this method is based on the propositions that:

(a) glycerol is mainly derived from the complete breakdown of triglyceride.

(b) glycerol is not metabolized to a significant extent in adipose tissue, since glycerokinase activity (an enzyme catalyzing the phosphorylation of glycerol to alpha-glycerophosphate) in rat adipose tissue is low.

(c) the rate of fatty acid synthesis de novo or fatty acid oxidation in adipose tissue is insignificant, therefore the net change in FFA in the incubation system represents only the balance between the rate of lipolysis and esterification [Vaughan, 1962; Steinberg and Vaughan, 1965].

These assumptions were re-evaluated in some aspects, when the balance method was applied to human adipose tissue [Lisch et al., 1973]. Studies with human adipose have provided evidence that the utilization of glycerol is insignificant, since

(a) glycerokinase activity is very low (only 2-3.5% of glycerol was phosphorylated [Koschinsky and Gries, 1971]).

(b) a small quantity of glycerol-1-14C was converted to CO2 and triglycerides in human adipose tissue under basal conditions [Björntorp, 1967b].
A more recent study [Arner et al., 1976] has shown that the amount of labelled glycerol converted to triglycerides is about 1000-fold less than that released to the incubation medium, both in the absence and in the presence of a maximally effective dose of isopropyl norepinephrine. Moreover, the study of Björntorp [1967b] showed that glycerol release into the incubation medium did not originate from the alpha-glycerophosphate pool, since no radioactive glycerol was detected on incubation of human adipose tissue with glucose-1-14C. In addition, the rate of oxidation of FFA in human adipose tissue seems to be low, as judged by the low consumption of oxygen by isolated fat cells [Galton and Bray, 1967]. Therefore, the application of balance method to human adipose tissue seems to be valid. Considering the fact that mono- and diglycerides are present in human adipose tissue [Hirsch and Goldrick, 1964; Björntorp et al., 1969b; Arner and Östman, 1974; Arner et al., 1976], the contribution of FFA which originate from these partial glycerides may affect the calculated rate of esterification. The latter parameter is underestimated if the hydrolysis of partial glycerides is not taken into account. This argument is weakened by the studies of Arner et al. [1976], who showed that there was no difference in the mean concentration of partial glycerides in human adipose tissue either before or after 2 hour basal incubation. This finding indicates no accumulation of partial glycerides present in the tissue during basal lipolysis. The hydrolysis of partial glycerides (if any occurs) seems to be unimportant in the estimation of esterification of FFA by the balance method in the basal conditions, since a negative re-esterification rate was not recorded in the study of Lisch et al. [1973] and in the present work. However, the diglyceride content in human adipose tissue was raised in maximally stimulated lipolysis, since
diglyceride lipase was also a rate-limiting enzyme in the lipolytic process [Arner et al., 1976]. The contribution of diglyceride hydrolysis may be of importance in evaluating the re-esterification rate of FFA in the condition of elevated lipolysis.

In contrast, the metabolism of glycerol in adipose tissue seems to be of some significance in causing an underestimation of the rate lipolysis in vitro. This follows from preliminary observations by Dr. R.B. Goldrick. He has shown that under optimal conditions there is sufficient glycerokinase present in human subcutaneous fat to rephosphorylate 15.0% of the glycerol produced under basal conditions in vitro. However, his subsequent investigations have failed to show any changes in glycerokinase activity in subcutaneous adipose tissue during starvation or overfeeding with fat or carbohydrate. Thus, although glycerol production may well underestimate lipolysis, the error is a systematic one and does not invalidate serial measurements of glycerol in testing the effects of dietary manipulations on lipolysis or esterification.

Serial studies of metabolism of subcutaneous adipose tissue in this study were achieved by a needle biopsy technique. Adipose tissue biopsies were successively performed about 6-7 times in 3 weeks in normal weight subjects without any complications. Trauma of adipose tissue may reduce basal metabolic activity and the effect of insulin on glucose metabolism in adipose tissue [Hirsch and Goldrick, 1964; Björntorp and Martinsson, 1967]. This problem can be counted as a systematic error, since the same technique was used to obtain the specimens throughout the study period. Moreover, subjects themselves were their own controls.

The metabolic parameters of adipose tissue in this work were
expressed in terms of metabolic activity per cell basis, which is suggested to be an appropriate reference in comparison with the metabolism of adipose tissue from different individuals [Salans et al., 1968; Gries et al., 1972; Pykäläistö et al., 1975a]. This is because the metabolic activity of adipose tissue is a direct function of the size of the fat cell (see Chapter 1, Section 1.1c). The other reference values (adipose tissue wet weight, triglyceride content, protein and DNA contents) are inappropriate in the interpretation of the results of adipose tissue obtained from different subjects. The number of adipose cells per wet weight of tissue is highly variable depending on the size of individual fat cells. The diameter of the fat cell varies greatly in different individuals and depends on the amount of triglycerides per cell. Therefore, the adipose tissue triglyceride content gives no real information on the number of adipose cells. Deoxyribonucleic acid (DNA) content represents nuclear material from adipose tissue. However, study with rat adipose tissue has shown that only 40% of the adipose tissue DNA arises from adipose cells [Rodbell, 1964a]. The adipose tissue DNA is an inexact estimation of adipose cell number. The same comments apply to adipose tissue protein, since on the average only 50% of the tissue protein comes from fat cells [Rodbell, 1964a].

The purpose of introducing various dietary regimes to the subjects is to examine adipose tissue metabolism in response to changes in dietary composition (carbohydrate or fat) and caloric intake (overfeeding or starvation). The reason for studying metabolic activities in adipose tissue after the standard high carbohydrate breakfast will be discussed in Chapter 3 (Effects of hypercaloric, eucaloric and hypercaloric diets on lipolysis esterification, and tissue free fatty acid concentrations in human adipose tissue), Section 3.4.
Subjects participating in the studies of the effects of dietary changes on adipose tissue metabolism (Chapter 3) comprised both normal weight and overweight subjects, some of whom had hypertriglyceridaemia or combined hyperlipidaemia or mild diabetes mellitus. They were included in different groups of dietary manipulations, since there were no differences in response of adipose tissue to a particular dietary program within the group of subjects. The data obtained during feeding with a eucaloric diet or experimental diets in the same group of subjects (either normal, obese, hypertriglyceridaemic or diabetic) were pooled for statistical analysis.

In Chapter 4, the effects of overfeeding on carbohydrate tolerance and insulin secretion were measured in young, lean, healthy adults. The responses of metabolites in blood (glucose, insulin, glycerol and FFA) were observed following ingestion of the standard high carbohydrate breakfast. The reason for using this meal instead of a conventional glucose tolerance test are:

(a) the adipose tissue metabolism (in Chapter 3) was observed $1\frac{1}{2}$ hours after consumption of the former meal. An exact explanation for the different effects of fat and glucose overfeeding on the metabolism of adipose tissue in vivo can not be given unless the metabolic events which occur in vivo are also assessed;

(b) in fact, the conventional oral carbohydrate tolerance can be used to examine the effects of overfeeding in vivo, but the metabolic responses following ingestion of a standard meal are more physiological than those observed after an acute glucose load [Reaven et al., 1972].
CHAPTER 3

EFFECTS OF HYPOCALORIC, EUCALORIC AND HYPERCALORIC DIETS ON LIPOLYSIS, ESTERIFICATION AND TISSUE FREE FATTY ACID CONCENTRATIONS IN HUMAN ADIPOSE TISSUE

3.1 INTRODUCTION

The effects of dietary factors on the metabolism of human adipose tissue in vitro have been studied by a number of investigators. With the balance technique of Vaughan [1962] it has been shown that prolonged starvation of obese subjects increases lipolysis in adipose tissue but has no effect on the re-esterification of FFA [Kjellberg and Östman, 1971]. However, studies using glucose-U-¹⁴C or palmitate-1-¹⁴C have shown greatly reduced rates of esterification of FFA by adipose tissue during prolonged starvation in obese and non-obese subjects [Goldrick and Hirsch, 1964]. Isocaloric substitution of carbohydrate for dietary fat stimulates glyceride-glycerol synthesis from glucose and glucose oxidation, and also enhances the effects of insulin on human adipose tissue in vitro [Hirsch and Goldrick, 1964; Salans et al., 1974]. Unlike the situation in rat adipose tissue [Hausberger and Milstein, 1955], stimulation of fatty acid synthesis has not been a consistent finding in human adipose tissue during the feeding of high carbohydrate diets [Hirsch and Goldrick, 1964; Salans et al., 1974]. Nevertheless, increased glyceride-glycerol synthesis indicates an accelerated rate of uptake and esterification of FFA; and this would represent a useful adaptation to the hypertriglyceridaemia so commonly associated with the
exhibition of high carbohydrate diets in man [Farquhar et al., 1966]. On the other hand, any changes in esterification of FFA may be offset by increased rates of basal lipolysis which have been reported in adipose tissue from individuals fed diets enriched with sucrose [Smith et al., 1973]. There is no information as to whether isocaloric substitution of carbohydrate for dietary fat induces a net uptake and esterification of FFA or simply increases the rate of turnover of adipose tissue triglylyceride fatty acids. Isocaloric substitution of fat for dietary carbohydrate depresses basal and insulin stimulated glucose oxidation and fatty acid synthesis from pyruvate but has no effect on basal lipolysis in human adipose tissue [Sims et al., 1973]. Similar findings have been reported in rat adipose tissue after feeding diets enriched with fat [Hausberger and Milstein, 1955; Ogundipe and Bray, 1974; Smith et al., 1974]. However, it is not known whether isocaloric substitution of fat for carbohydrate in the diet has any effect on the uptake of FFA by adipose tissue in man. Furthermore, there is virtually no information on the effects of overfeeding fat or carbohydrate on the metabolism of human adipose tissue. Elegant clinical studies have been performed before and after the induction of obesity [Sims et al., 1973; Salans et al., 1974], but as far as can be ascertained only one investigation has been carried out during weight gain [Sjöström, 1973b]. The latter involved 6 obese females who consumed for 5 days an additional 1,000 calories/day in the form of a high carbohydrate formula diet. Overfeeding stimulated activity of enzymes for de novo fatty acid synthesis in adipose tissue, but had no effect on fatty acid synthesis from glucose in the presence of insulin. Increased basal plasma insulin and triglyceride concentrations and decreased basal level of plasma cholesterol were observed during overfeeding [Sjöström, 1973b]. Since de novo fatty acid synthesis in adipose tissue is generally accepted to
play only a minor role in fat deposition in man [Björntorp et al., 1971a; Sjöström, 1973b] further studies are required to elucidate the mechanism whereby human adipose tissue stores triglyceride in response to overfeeding. In the present investigation, rates of lipolysis and esterification have been measured (by a modification of the balance method of Vaughan) in serial biopsies of human adipose tissue taken before and during the administration of eucaloric, hypocaloric and hypercaloric diets.

3.2 MATERIALS AND METHODS

Of 31 subjects studied, 16 subjects were lean and 15 subjects were obese. Their relevant clinical data and values of adipose cell weight are shown in Table 3.1. Four grossly obese subjects (Nos. 2, 24, 25, 31) had mild untreated diabetes mellitus: six subjects (Nos. 5, 9, 12, 14, 24, 31) were hypertriglyceridaemic and subjects 2 and 18 had combined hyperlipidaemia. The remaining subjects were either healthy student volunteers or obese patients without evidence of any associated metabolic disorder. (See other details for experimental condition in Section 2.1).

Experimental Design

Study I (subjects Nos. 1-4) and Study II (subjects Nos. 5, 6) were preliminary experiments designed to examine the effects of meal feeding on the metabolism of adipose tissue. In both studies, all subjects were maintained for 4 days on a standard eucaloric diet (P:F:C=15:39:46%). The protocol for Study I was as follows. On the fifth day following a 14 hour fast, 1 or 2 adipose tissue biopsies were performed between 7.45
### Table 3.1
Clinical Data

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<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>%IBW*</th>
<th>Fat Cell Size (μg TO/cell)</th>
<th>Glucose (mg/100 ml)</th>
<th>Cholesterol (mg/100 ml)</th>
<th>Triglycerides (mg/100 ml)</th>
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* %IBW = per cent ideal body weight.
+ μg triolein/adipose cell.
# The designations "a" and "b" refer to the status of subject 19 at the beginning of Studies V and VI respectively.
and 8.15 a.m. A eucaloric high carbohydrate diet (P:F:C = 12:16:72%) was then consumed over a period of 20 minutes in 3 equal portions at 8.30 a.m., 12.30 p.m. and 4.30 p.m. Single adipose tissue biopsies were performed at 9.30 a.m., 11.00 a.m., 2.00 p.m. and 6.00 p.m. On the following day after a 14 hour fast, another adipose tissue biopsy was performed.

In Study II, the same protocol as used in Study I was followed except that a eucaloric high saturated fat diet (P:F:C = 10:71:19%) was fed in 3 equal portions.

**Study III-IV.** For the first 4-10 days all subjects were maintained on the standard eucaloric diet (P:F:C = 15:39:46%). The latter diet had an average ratio of polyunsaturated/saturated fatty acids (P/S ratio) of 0.08. Adipose tissue biopsies were performed 2 to 3 times per week, 1½ hours after a standard high carbohydrate breakfast (P:F:C = 8:25:67% calories = 750) which was fed daily. In Study III, subjects Nos. 7-14 were changed to a eucaloric high carbohydrate diet (P:F:C = 13:13:74%) for 4-6 days. The average daily intake of cholesterol was 460 mg on the standard diet and 181 mg on the high carbohydrate diet. In Study IV, subjects Nos. 8, 10, 13 and 14 who had participated in Study III, were maintained on the eucaloric high carbohydrate diet for a further 4 days, during which time they received an additional 30% of calories as glucose. Subject No. 15 was also included in Study IV. However, she was changed from the standard to the hypercaloric diet for 9 days without an intervening period of eucaloric high carbohydrate feeding. The average final composition of the hypercaloric diet was P:F:C = 11:10:79%. The mean cholesterol intake per day was 498 mg on the standard diet and 200 mg on the hypercaloric high carbohydrate diet. Study V involved subjects Nos. 16-19. The designation 19a in Table 3.1 refers to the status of subject 19 at the beginning of this study. The standard
eucaloric diet was fed throughout Study V, but during the final 8 (subjects Nos. 16, 17) and 12 (subjects Nos. 18, 19a) days, an additional 50% of calories were fed predominantly as saturated fat. The average final composition of the hypercaloric high fat diet was P:F:C = 11:56:33% with a mean P/S ratio of 0.14. The daily intake of cholesterol averaged 478 mg on the standard eucaloric diet and 868 mg during overfeeding with saturated fat. Study VI involved subjects 19b and 20. The designation 19b in Table 3.1 refers to the status of subject 19 at the commencement of this study which was 3 months after he had participated in Study V. The standard eucaloric diet was fed throughout Study VI, but during the final 14 days, an additional 50% of calories were fed as polyunsaturated fat. The average final composition of this hypercaloric high fat diet was P:F:C = 11:57:32% with a mean P/S ratio of 1.26. The daily intake of cholesterol averaged 522 mg in both dietary periods.

In order to examine the effects of these dietary manipulations on the metabolism of adipose tissue, adipose tissue biopsies were also performed twice a week. The tissue was removed 1½ hours after the standard high carbohydrate breakfast which was given daily throughout the study period. This meal provided the quantity of calories and compositions identical to those given to subjects during maintaining on the eucaloric standard diet.

Study VII was performed on 6 very obese subjects with the object of inducing an average deficit of 3,150 calories per day. Subjects were maintained on a standard eucaloric (P:F:C = 14:39:47%) for 4-10 days. These subjects were then submitted to a caloric restriction regimen. This was effected by fasting subject No. 7 for one week, providing 220 to 640 calories per day to subjects Nos. 21, 22 and 24 for 2 weeks, and
690 calories per day to subject 25 for one week. Subject No. 23 was placed on 550 calories diet per day for 3 weeks.

Study VIII was performed on 2 non-obese subjects (Nos. 26, 27) in whom deficits of 2,400 and 1,900 calories per day for 8 days were induced by reducing the standard eucaloric diet to 600 and 300 calories per day respectively.

In Studies VII and VIII adipose tissue biopsies were performed at 8.30 a.m. after a fast of at least 14 hours, whilst taking the eucaloric standard diet, and at the same time on the restriction of caloric intake.

In Studies III-VIII, blood samples were taken every week day following an overnight fast for determinations of plasma glucose, insulin, cholesterol, triglycerides, glycerol and FFA concentrations.

Subjects Nos. 28-31 were placed on only the standard eucaloric diet for one week. Adipose tissue biopsies were performed 3 times to study metabolism of adipose tissue in the eucaloric state, following a 14 hour fast. Data obtained were included in the study of inter-relationships between adipose cell weight and parameters of metabolism in adipose tissue. Blood specimens were also collected every week-day (subjects Nos. 28-30) for estimations of the same plasma constituents, as measured in Studies III-VIII.

Specific details of caloric intake and dietary composition are demonstrated in Appendix 1. Procedures for adipose tissue biopsy, studies of metabolic activities in adipose tissue (lipolysis, esterification, tissue FFA concentration) and plasma analyses are described in Materials and Methods (Chapter 2).
3.3 RESULTS

(a) Studies I and II: Effects of High Carbohydrate and High Fat Meals

The data obtained in these studies are shown in Table 3.2. In the postabsorptive state, adipose tissue obtained from the 4 grossly obese subjects selected for Study I released FFA into the incubation medium and at the same time showed a reduction in the concentration of tissue FFA. The release of FFA exceeded the disappearance of tissue FFA so that there was a net accumulation of FFA in the incubation system. Under these conditions, lipolysis exceeded esterification. The data obtained from the serial biopsies on these 4 subjects following the ingestion of the high carbohydrate meal showed no systematic differences after breakfast, lunch and dinner so the data recorded in the absorptive state were pooled for analysis in Table 3.2. Under the latter conditions the concentrations of tissue FFA decreased at the same rate in vitro as in the postabsorptive state. Here also, the release of FFA exceeded the changes in tissue FFA so causing a net accumulation of FFA in the incubation system. However, the net change in FFA was significantly less than that recorded in the postabsorptive state, the difference being solely attributable to a 30% reduction in the rate of FFA release. No significant changes in lipolysis or esterification were demonstrable following the feeding of the high carbohydrate meal. In Study II, adipose tissue obtained in the postabsorptive state from 2 other grossly obese subjects showed the same rate of decline in tissue FFA as the subjects selected for Study I. However, in Study II the rate of FFA release was slightly less than the rate of disappearance of tissue FFA and a very small negative net change in the FFA content of the system was observed. Lipolysis and esterification were therefore
Table 3.2
Effects of high carbohydrate and high fat meals on lipolysis and esterification in vitro.*

<table>
<thead>
<tr>
<th></th>
<th>Postabsorptive State</th>
<th>Absorptive State</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>μmoles FFA/10⁶ adipose cells/2 hours</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Study I</strong> †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipolysis</td>
<td>3.96 ± 0.57</td>
<td>3.02 ± 0.27</td>
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<tr>
<td>Esterification</td>
<td>2.08 ± 0.41</td>
<td>1.83 ± 0.24</td>
</tr>
<tr>
<td>Net change in FFA</td>
<td>1.88 ± 0.46</td>
<td>1.19 ± 0.19</td>
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<tr>
<td>FFA release (M₂hr - M₀)</td>
<td>2.30 ± 0.49</td>
<td>1.62 ± 0.15</td>
</tr>
<tr>
<td>Change in tissue FFA (T₂hr - T₀)</td>
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<td>-0.43 ± 0.11</td>
</tr>
<tr>
<td><strong>Study II</strong> †</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipolysis</td>
<td>2.02 ± 0.49</td>
<td>2.60 ± 0.59</td>
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<tr>
<td>Esterification</td>
<td>2.13 ± 0.36</td>
<td>2.37 ± 0.53</td>
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<tr>
<td>Net change in FFA</td>
<td>-0.11 ± 0.31</td>
<td>0.23 ± 0.26</td>
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<td>FFA release (M₂hr - M₀)</td>
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<td>0.80 ± 0.35</td>
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<tr>
<td>Change in tissue FFA (T₂hr - T₀)</td>
<td>-0.42 ± 0.29</td>
<td>-0.57 ± 0.26</td>
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</tbody>
</table>

* Values are given as the means ±1 S.E.M. for subjects Nos. 1-4 in Study I and for subjects Nos. 5 and 6 in Study II.
† In Study I a eucaloric high carbohydrate diet (P:F:C = 12:16:72% calories) and in Study II a eucaloric high fat diet (P:F:C = 10:71:19% calories) was given in 3 equal portions at 8.30 a.m., 12.30 p.m. and 4.30 p.m. In each subject 1-2 adipose tissue biopsies were performed in the postabsorptive state between 7.45 and 8.15 a.m. on the day of this study and at the same time on the following day. Single biopsies were performed in the absorptive state at 9.30 a.m., 11.00 a.m., 2.00 p.m. and 6.00 p.m. The data shown in the absorptive state are derived from the pooled values obtained at 9.30 a.m., 11.00 a.m., 2.00 p.m. and 6.00 p.m.
# Indicates a P value < 0.05 obtained by comparing values in the postabsorptive state with those in the absorptive state.

almost in balance. In Study II the data obtained following the ingestion of the high fat meal showed no systematic differences after breakfast, lunch and dinner. Hence the data recorded in the absorptive state have been pooled for analysis in Table 3.2. No significant
changes in adipose tissue metabolism were demonstrable following the ingestion of the high fat meal. On the basis of these preliminary studies, adipose tissue biopsies were performed in studies III-VI 1.5 hours following a standard high carbohydrate breakfast (P:F:C = 8:25:67%) of similar composition to the meal used in Study I (P:F:C = 12:16:72%). The high carbohydrate breakfast was better tolerated on a long term basis than the high fat meal. The use of a standard breakfast enabled a direct comparison to be made of the effects of isocaloric or hypercaloric diets or different dietary compositions on the metabolism of adipose tissue. Since this meal was fed daily, any changes in response to meal represent the effects of antecedent dietary manipulations at lunch and dinner. This approach however, was inappropriate for examining the effects of hypocaloric diets; and in Studies VII-VIII adipose tissue biopsies were routinely obtained in the postabsorptive state.

(b) Study III: Effects of Isocaloric High Carbohydrate Diet

Isocaloric substitution of carbohydrate for dietary fat was performed in 3 very obese women (subjects Nos. 7, 11, 12), one mildly obese man (subject No. 10) and 4 lean subjects, 2 of whom were hypertri-glyceridaemic (subjects Nos. 9, 14). No differences in adipose tissue response were observed so the data were pooled and are listed with the data obtained from the plasma analyses in Table 3.3. The high carbohydrate diet elevated the basal plasma triglycerides and lowered the plasma cholesterol in all subjects. The latter effect may have been partly caused by the low cholesterol content of the high carbohydrate diet [Connor et al., 1964]. No significant changes in the post-absorptive plasma concentrations of glycerol, FFA, glucose or insulin were observed. In contrast to its effects on the plasma cholesterol and
**Study III: Effects of isocaloric substitution of carbohydrate for dietary fat.***

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (kg)</strong></td>
<td>89.1 ± 3.10</td>
<td>89.5 ± 3.40</td>
</tr>
<tr>
<td><strong>Cholesterol (mg/100 ml)</strong></td>
<td>193 ± 4</td>
<td>175 ± 4</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/100 ml)</strong></td>
<td>142 ± 8</td>
<td>189 ± 15</td>
</tr>
<tr>
<td><strong>Glycerol (µmoles/l)</strong></td>
<td>81 ± 8</td>
<td>93 ± 9</td>
</tr>
<tr>
<td><strong>FFA (µmoles/l)</strong></td>
<td>554 ± 36</td>
<td>553 ± 37</td>
</tr>
<tr>
<td><strong>Insulin (µu/ml)</strong></td>
<td>15.7 ± 0.9</td>
<td>20.6 ± 5.1</td>
</tr>
<tr>
<td><strong>Glucose (mg/100 ml)</strong></td>
<td>82 ± 2</td>
<td>79 ± 1</td>
</tr>
<tr>
<td><strong>Basal Plasma Concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lipolysis</strong></td>
<td>1.90 ± 0.16</td>
<td>1.71 ± 0.13</td>
</tr>
<tr>
<td><strong>Esterification</strong></td>
<td>1.75 ± 0.13</td>
<td>1.68 ± 0.15</td>
</tr>
<tr>
<td><strong>Net change in FFA</strong></td>
<td>0.15 ± 0.15</td>
<td>0.03 ± 0.18</td>
</tr>
<tr>
<td><strong>FFA release (M$<em>{2hr}$ - M$</em>{0}$)</strong></td>
<td>0.50 ± 0.12</td>
<td>0.35 ± 0.14</td>
</tr>
<tr>
<td><strong>Change in tissue FFA (T$<em>{2hr}$ - T$</em>{0}$)</strong></td>
<td>-0.35 ± 0.05</td>
<td>-0.32 ± 0.07</td>
</tr>
</tbody>
</table>

*Values are given as means ±1 S.E.M. for subjects Nos. 7 to 14 in Study III.

†, ‡ Indicate P values < 0.05 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and high carbohydrate diets.

Triglycerides the eucaloric high carbohydrate diet had no significant effect on any of the parameters measured in adipose tissue 1½ hours after the standard meal. This was so when the data were examined on a day-to-day basis and also when the data obtained on the high carbohydrate diet were pooled for analysis (Table 3.3). In both dietary periods, FFA disappeared from the tissues and accumulated in the incubation medium at approximately equal rates. The net changes in FFA content of the
incubation system were thus very small and lipolysis and esterification were almost in balance. Day-to-day variations in lipolysis and esterification were calculated from the residual sums of squares in the analysis of variance for the data in Table 3.3. The coefficients of variation for lipolysis and esterification were 13.2% and 14.5% respectively and represent the biological and methodological errors involved in estimating these parameters.

(c) Study IV: Effects of Overfeeding with Glucose

The effects of supplementing the eucaloric high carbohydrate diet with glucose are shown in Table 3.4. Overfeeding with glucose caused a small but significant increase in body weight. The plasma triglycerides rose and the plasma cholesterol fell to a greater extent than on the eucaloric high carbohydrate diet. The reduction in plasma cholesterol can not be explained by differences in dietary cholesterol between Studies III and IV. It presumably reflects greater changes in the composition and concentrations of plasma lipoproteins when the high carbohydrate diet was supplemented with glucose [Schonfeldt et al., 1976]. Additional findings were a significant reduction in the postabsorptive concentrations of plasma FFA and a rise in the basal plasma insulin. Plasma glucose and glycerol concentrations were unaffected by the hypercaloric high carbohydrate diet. The reduction in plasma FFA without any change in plasma glycerol suggests that esterification in adipose tissue in the postabsorptive state was increased by overfeeding with glucose, possibly mediated by the rise in basal plasma insulin [Goldrick, 1967a]. In the eucaloric state, the findings in adipose tissue were similar to those in Study III. Thus, FFA disappeared from the tissues and were released into the incubation medium at almost equal rates so that only a
Table 3.4
Study IV: Effects of overfeeding with glucose.*

<table>
<thead>
<tr>
<th></th>
<th>Diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eucaloric High Carbohydrate plus 30% of Calories as Glucose</td>
</tr>
<tr>
<td></td>
<td>P:F:C (15:39:46%)</td>
</tr>
<tr>
<td></td>
<td>P:F:C (11:10:79%)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>77.3 ± 3.20</td>
</tr>
<tr>
<td></td>
<td>78.5 #</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>190 ± 6</td>
</tr>
<tr>
<td></td>
<td>156 ± 5</td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>128 ± 11</td>
</tr>
<tr>
<td></td>
<td>206 ± 21</td>
</tr>
<tr>
<td>Glycerol (μmoles/l)</td>
<td>59 ± 6</td>
</tr>
<tr>
<td></td>
<td>62 ± 8</td>
</tr>
<tr>
<td>FFA (μmoles/l)</td>
<td>484 ± 25</td>
</tr>
<tr>
<td></td>
<td>420 ± 26</td>
</tr>
<tr>
<td>Insulin (μu/ml)</td>
<td>16.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>23.4 ± 2.4</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>84 ± 2</td>
</tr>
<tr>
<td></td>
<td>83 ± 2</td>
</tr>
</tbody>
</table>

μmoles FFA/10⁶ Adipose Cells/2 hours

<table>
<thead>
<tr>
<th></th>
<th>Lipolysis</th>
<th>Esterification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.61 ± 0.13</td>
<td>1.47 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>1.71 ± 0.18</td>
<td>2.32 ± 0.27</td>
</tr>
<tr>
<td>Net change in FFA</td>
<td>-0.10 ± 0.22</td>
<td>-0.85 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>0.43 ± 0.13</td>
<td>-0.04 ± 0.12</td>
</tr>
<tr>
<td>FFA release (M₂hr-M₀)</td>
<td>-0.53 ± 0.15</td>
<td>-0.81 ± 0.10</td>
</tr>
<tr>
<td>Change in tissue FFA (T₂hr-T₀)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Values are given as means ±1 S.E.M. for subjects Nos. 8, 10, 13-15 in Study IV.
† Body weight on the hypercaloric diet is the mean of the last recorded body weight in the 5 subjects.
‡ Indicated P values < 0.05 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and hypercaloric diets.

small net decrease in the FFA content of the system occurred.

Esterification and lipolysis were almost in balance. When the eucaloric high carbohydrate diet was supplemented with glucose, there was a marked increase in the rate of disappearance of FFA from the incubation system.

This was due to an inhibition of FFA release without any significant
change in the rate of decrease in tissue FFA. Lipolysis was unaffected and esterification was increased by 36%. Thus, overfeeding with glucose potentiated the response of adipose tissue to the high carbohydrate breakfast in such a way as to promote the uptake of FFA. This effect seems specific for overfeeding with glucose and does not simply represent the more prolonged period of high carbohydrate feeding in subjects Nos. 8, 10, 13 and 14 who were overfed after completing Study III. Subject No. 15 had no intervening period on the eucaloric high carbohydrate diet also exhibited increased esterification and inhibition of FFA release when transferred to the hypercaloric diet.

(d) Study V: Effects of Overfeeding with Saturated Fat

The results of this study are shown in Table 3.5. Supplementing the standard eucaloric diet with saturated fat had no effect on the plasma cholesterol concentration in spite of an average increase in dietary cholesterol of 390 mg/day. The postabsorptive concentrations of plasma triglycerides, FFA insulin and glucose were also unaffected. However, the plasma glycerol was elevated, suggesting an increased rate of lipolysis in the postabsorptive state. The response of adipose to the standard high carbohydrate breakfast was altered by overfeeding with saturated fat. Lipolysis was increased by 29% and FFA (which were taken up from the incubation medium in the eucaloric state) were released from the tissues during overfeeding. These changes in FFA uptake and release occurred without any alteration in the rate of disappearance of FFA from the tissues or in the rate of esterification. The latter exceeded lipolysis in both phases of the study so maintaining a net disappearance of FFA from the incubation system in spite of the changes in lipolysis during overfeeding.
Table 3.5
Study V: Effects of overfeeding with saturated fat.*

<table>
<thead>
<tr>
<th>Diets</th>
<th>Standard</th>
<th>Standard Eucaloric plus 50% of Calories as Saturated Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P:F:C (15:39:46%)</td>
<td>P:F:C (11:56:33%)</td>
</tr>
<tr>
<td>Body weight (kg)†</td>
<td>70.2 ± 1.10</td>
<td>72.8#</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>230 ± 7</td>
<td>223 ± 6</td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>118 ± 13</td>
<td>101 ± 12</td>
</tr>
<tr>
<td>Glycerol (μmoles/l)</td>
<td>35 ± 2</td>
<td>47 ± 3#</td>
</tr>
<tr>
<td>FFA (μmoles/l)</td>
<td>440 ± 25</td>
<td>412 ± 31</td>
</tr>
<tr>
<td>Insulin (μu/ml)</td>
<td>11.8 ± 1.2</td>
<td>13.0 ± 1.1</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>89 ± 2</td>
<td>92 ± 2</td>
</tr>
<tr>
<td>Basal Plasma Concentrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipolysis</td>
<td>1.85 ± 0.17</td>
<td>2.38 ± 0.21#</td>
</tr>
<tr>
<td>Esterification</td>
<td>2.54 ± 0.37</td>
<td>2.73 ± 0.25</td>
</tr>
<tr>
<td>Net change in FFA (M_{2hr} - M_0)</td>
<td>-0.69 ± 0.40</td>
<td>-0.35 ± 0.24</td>
</tr>
<tr>
<td>FFA release (M_{2hr} - M_0)</td>
<td>-0.19 ± 0.21</td>
<td>0.18 ± 0.21#</td>
</tr>
<tr>
<td>Change in tissue FFA (T_{2hr} - T_0)</td>
<td>-0.50 ± 0.29</td>
<td>-0.53 ± 0.20</td>
</tr>
</tbody>
</table>

Values are given as means ±1 S.E.M. for subjects Nos. 16-19a in Study V.

† Body weight on the hypercaloric diet is the mean of the last recorded body weight in the 4 subjects.

#, Indicate P values < 0.05, < 0.01 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and hypercaloric diets.

(c) Study VI: Effects of Overfeeding with Polyunsaturated Fat

This was undertaken to determine whether overfeeding with polyunsaturated fat had the same effects on the metabolism of adipose tissue as overfeeding with saturated fat. The results of Study VI are shown in
Table 3.6: Effects of Overfeeding with Polyunsaturated Fat.

<table>
<thead>
<tr>
<th>Diets</th>
<th>Standard Eucaloric plus 50% of Calories as Polyunsaturated Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>P:F:C (15:39:46%)</td>
<td>P:F:C (11:57:32%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Body weight (kg)</th>
<th>65.8 ± 0.9</th>
<th>69.6 #</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>180 ± 3</td>
<td>155 ± 3</td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>70 ± 8</td>
<td>56 ± 6 #</td>
</tr>
<tr>
<td>Glycerol (μmoles/l)</td>
<td>32 ± 4</td>
<td>38 ± 6</td>
</tr>
<tr>
<td>FFA (μmoles/l)</td>
<td>379 ± 23</td>
<td>357 ± 29</td>
</tr>
<tr>
<td>Insulin (μu/ml)</td>
<td>14.9 ± 0.7</td>
<td>15.0 ± 1.3</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>88 ± 1</td>
<td>93 ± 2 #</td>
</tr>
</tbody>
</table>

Basal Plasma Concentrations

<table>
<thead>
<tr>
<th>μmoles FFA/10⁶ Adipose Cells/2 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipolysis</td>
</tr>
<tr>
<td>Esterification</td>
</tr>
<tr>
<td>Net change in FFA</td>
</tr>
<tr>
<td>FFA release (M₂hr⁻¹-M₀)</td>
</tr>
<tr>
<td>Change in tissue FFA (T₂hr⁻¹-T₀)</td>
</tr>
</tbody>
</table>

* Values are given as means ± S.E.M. for subjects Nos. 19b and 20 in Study VI.

† Body weight on the hypercaloric diet is the mean of the last recorded body weight in the 2 subjects.

#, Indicate P values < 0.05 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and hypercaloric diets.

Table 3.6. Although this study was carried out on only two subjects, one of them, viz., subject 19, also participated in Study V. Overfeeding with polyunsaturated fats caused the basal plasma cholesterol and triglyceride concentrations to fall, and the basal plasma glucose to rise. These events occurred even when the intake of dietary cholesterol
during the control and overfeeding periods was comparable. In view of the well documented lipid lowering effects of polyunsaturated fats [Ahrens et al., 1957; Nestel, 1973; Nestel et al., 1974], the changes in plasma cholesterol and triglyceride were not unexpected. However, the reason for the rise in plasma glucose is unclear, its real significance remains to be established. In most other respects overfeeding with polyunsaturated fats produced similar changes to those observed in Study V. Thus, the plasma FFA and insulin concentrations were unaltered and the plasma glycerol levels tended to be elevated, although not significantly so during overfeeding. Furthermore, like Study V, lipolysis in adipose tissue was increased by 40%. Esterification was unaltered and remained in excess of lipolysis indicating a net utilization of FFA when the standard eucaloric diet was supplemented with polyunsaturated fat. In this particular instance, no effect on FFA release by adipose tissue was demonstrable. The small number of subjects investigated in Study VI prevents any generalization on the latter finding. As in Study V, there was no change in the rate of disappearance of FFA from the tissues during overfeeding. Thus, both Study V and Study VI showed that the effects on adipose tissue of overfeeding fat were different from those of overfeeding carbohydrate and were largely independent of the type of fat which used to supplement the standard eucaloric diet.

(f) Study VII: Effects of Semi-Starvation in Obese Subjects

The effects of hypocaloric diets on obese subjects are shown in Table 3.7. In this study, subjects were placed in negative caloric balance for 1 to 3 weeks. Changes in the metabolism of adipose tissue occurred within the first 2 days; and as they showed no further changes
Table 3.7

Study VII: Effects of semi-starvation in obese subjects.*

<table>
<thead>
<tr>
<th>Diets</th>
<th>Standard Eucaloric</th>
<th>Hypocaloric</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight (kg)</strong> †</td>
<td>129.2 ± 2.20</td>
<td>122.4 †</td>
</tr>
<tr>
<td><strong>Basal Plasma Concentrations</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>169 ± 7</td>
<td>152 ± 5 †</td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>145 ± 19</td>
<td>102 ± 6 †</td>
</tr>
<tr>
<td>Glycerol (μmoles/l)</td>
<td>92 ± 6</td>
<td>130 ± 14 †</td>
</tr>
<tr>
<td>FFA (μmoles/l)</td>
<td>527 ± 35</td>
<td>824 ± 33 †</td>
</tr>
<tr>
<td>Insulin (μu/ml)</td>
<td>24.4 ± 1.9</td>
<td>13.3 ± 1.0 †</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>95 ± 4</td>
<td>81 ± 3 †</td>
</tr>
<tr>
<td><strong>Lipolysis</strong></td>
<td>3.26 ± 0.32</td>
<td>5.66 ± 0.59 †</td>
</tr>
<tr>
<td><strong>Esterification</strong></td>
<td>3.01 ± 0.32</td>
<td>2.61 ± 0.37</td>
</tr>
<tr>
<td><strong>Net change in FFA</strong></td>
<td>0.25 ± 0.29</td>
<td>3.05 ± 0.50 †</td>
</tr>
<tr>
<td><strong>FFA release (M_{2hr} - M_{0})</strong></td>
<td>1.49 ± 0.27</td>
<td>3.49 ± 0.43 †</td>
</tr>
<tr>
<td><strong>Change in tissue FFA (T_{2hr} - T_{0})</strong></td>
<td>-1.24 ± 0.31</td>
<td>-0.44 ± 0.28 †</td>
</tr>
</tbody>
</table>

* Values are given as means ±1 S.E.M. for subjects Nos. 7, 21-25 in Study VII.
† Body weight on the hypocaloric diet is the mean of the last recorded body weight in the 6 subjects.
#, † Indicate P values < 0.05 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and hypocaloric diets.
# This low value was due to a large fall in plasma glucose in subject 24 and was not statistically significant.

with time, the data were pooled for comparison with those obtained on the standard eucaloric diet. Negative caloric balance induced a small but significant fall in the concentration of plasma cholesterol in all subjects and a larger fall in the plasma triglycerides. The plasma glycerol and FFA concentrations were increased by 41% and 56% respectively; and there was a marked fall in the previously elevated
concentrations of the basal plasma insulin. There was no significant change in the concentration of plasma glucose. Adipose tissue responded to negative caloric balance with a 74\% increase in the rate of lipolysis. There was no significant reduction in the rate of esterification. The release of FFA was increased more than two-fold and the rate at which FFA disappeared from the tissues was decreased to about one third of the values recorded in the eucaloric state. In the latter instance, lipolysis and esterification were almost in balance so that the net production of FFA was very small. During weight loss, lipolysis exceeded esterification by a factor of two with a corresponding increase in the net production of FFA. The findings in adipose tissue indicate that the elevated concentrations of plasma glycerol and FFA during semi-starvation reflected increased rates of lipolysis.

(g) Study VIII: Effects of Semi-Starvation in Non-obese Subjects

The responses of the two non-obese subjects in Study VIII to semi-starvation are shown in Table 3.8. There was no significant change in the concentration of plasma cholesterol, glycerol or insulin during semi-starvation, whereas the plasma FFA levels rose and the plasma glucose and triglyceride concentrations fell. The failure to demonstrate a rise in the plasma glycerol may well reflect the small number of subjects studied and the smaller caloric deficit in Study VIII rather than any real difference in response between obese and non-obese individuals. Thus, lipolysis in adipose tissue was increased by 68\%, esterification was unaffected and the net accumulation of FFA in the incubation system was increased when adipose tissue biopsies were obtained from non-obese subjects during weight loss. FFA release from adipose tissue showed the same qualitative changes in the non-obese as
Table 3.8
Study VIII: Effects of semi-starvation in non-obese subjects.*

<table>
<thead>
<tr>
<th></th>
<th>Standard Eucaloric</th>
<th>Hypocaloric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>70.22 ± 1.16</td>
<td>66.85#</td>
</tr>
<tr>
<td>Cholesterol (mg/100 ml)</td>
<td>210 ± 4</td>
<td>202 ± 5</td>
</tr>
<tr>
<td>Triglycerides (mg/100 ml)</td>
<td>80 ± 9</td>
<td>63 ± 7#</td>
</tr>
<tr>
<td>Glycerol (µmoles/l)</td>
<td>47 ± 5</td>
<td>61 ± 8</td>
</tr>
<tr>
<td>FFA (µmoles/l)</td>
<td>559 ± 43</td>
<td>855 ± 68#</td>
</tr>
<tr>
<td>Insulin (µu/ml)</td>
<td>13.4 ± 1.0</td>
<td>11.4 ± 0.9</td>
</tr>
<tr>
<td>Glucose (mg/100 ml)</td>
<td>83 ± 2</td>
<td>73 ± 3#</td>
</tr>
</tbody>
</table>

µmoles FFA/10^6 Adipose Cells/2 hours

<table>
<thead>
<tr>
<th></th>
<th>Standard Eucaloric</th>
<th>Hypocaloric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipolysis</td>
<td>1.58 ± 0.25</td>
<td>2.66 ± 0.37#</td>
</tr>
<tr>
<td>Esterification</td>
<td>1.75 ± 0.52</td>
<td>1.47 ± 0.26#</td>
</tr>
<tr>
<td>Net change in FFA</td>
<td>-0.17 ± 0.42</td>
<td>1.19 ± 0.31#</td>
</tr>
<tr>
<td>FFA release (M_2hr-M_0)</td>
<td>0.18 ± 0.19</td>
<td>0.96 ± 0.33</td>
</tr>
<tr>
<td>Change in tissue FFA (T_2hr-T_0)</td>
<td>-0.35 ± 0.24</td>
<td>0.23 ± 0.20</td>
</tr>
</tbody>
</table>

* Values are given as mean ±1 S.E.M. for subjects Nos. 26 and 27 in Study VIII.
† Body weight on the hypocaloric diet is the mean of the last recorded body weight in the 2 subjects.
#, Indicate P values < 0.05, < 0.01 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and hypocaloric diets.

in the obese during semi-starvation but the differences just failed to reach significance. One possible difference in response between the subjects in Studies VII and VIII was a rise in the concentration of tissue FFA during incubation of adipose tissue from non-obese subjects during weight loss (Table 3.8). This was the only instance in which the tissue FFA concentrations increased when adipose tissue was incubated.
Table 3.9
Effects of dietary changes on tissue FFA concentrations ($T_0$ FFA) after 30 minutes of preincubation.*

<table>
<thead>
<tr>
<th>Study</th>
<th>µmoles FFA/10⁶ Adipose Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Postabsorptive State</td>
</tr>
<tr>
<td>I</td>
<td>4.61 ± 0.67</td>
</tr>
<tr>
<td>II</td>
<td>7.77 ± 1.22</td>
</tr>
<tr>
<td></td>
<td>Standard Eucaloric Diet</td>
</tr>
<tr>
<td>III</td>
<td>2.48 ± 0.22</td>
</tr>
<tr>
<td>IV</td>
<td>4.90 ± 1.46</td>
</tr>
<tr>
<td>V</td>
<td>12.47 ± 1.26</td>
</tr>
<tr>
<td>VI</td>
<td>8.12 ± 0.97</td>
</tr>
<tr>
<td>VII</td>
<td>21.86 ± 3.23</td>
</tr>
<tr>
<td>VIII</td>
<td>4.87 ± 1.54</td>
</tr>
</tbody>
</table>

* Values are given as means ± 1 S.E.M. for the studies listed in Tables 3.2-3.8.

†, #, & indicate P values < 0.05 and < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard and test diets. Comparisons were made by the analysis of variance.

A significant interaction (P < 0.001) between subjects and diet in the analysis of variance indicated a fall in tissue FFA concentration in 4 of the 5 subjects when fed the hypercaloric high carbohydrate diet.

(h) Tissue FFA Concentrations ($T_0$ FFA)

Data of tissue FFA ($T_0$) concentrations were also examined when calculating net change in FFA in the incubation system. It was evident that dietary manipulations altered the $T_0$ FFA concentrations as shown in Table 3.9. In Studies I and II both the high carbohydrate and the high fat meals caused a small, but significant reduction in $T_0$ FFA indicating that the composition of the meal was unimportant in this respect. The fact that such a change was observed in Study II is of particular interest because it will be recalled that the high fat meal did not affect any of the other parameters measured in adipose tissue. Even
though the $T_0$ FFA were determined after a 30 minute preincubation in vitro, it is reasonable to suppose that meal feeding lowered the concentration of tissue FFA in vivo. In Study III the eucaloric high carbohydrate diet had no effect on $T_0$ FFA over and above that produced by the standard high carbohydrate breakfast. On the other hand, in Study IV overfeeding with glucose augmented the effects of the high carbohydrate breakfast in 4 of the 5 subjects. Overfeeding with saturated fat (Study V) had similar effects to overfeeding with glucose, and these were progressive insofar as the $T_0$ FFA concentrations averaged 10.46, 5.59 and 4.04 µmoles/10^6 adipose cells respectively on the third, seventh and eighth days of overfeeding. In Study VI overfeeding with polyunsaturated fat failed to augment the effects of the standard breakfast on the $T_0$ FFA when the data for the overfeeding period were pooled. However, breakdown analysis showed that the average values for $T_0$ FFA obtained during the second week of overfeeding (4.33 µmoles/10^6 adipose cells) were significantly lower ($P < 0.01$) than those recorded in the eucaloric state. Thus, the $T_0$ FFA concentrations were reduced to a small extent by meal feeding and this response was exaggerated when subjects were in a state of positive caloric balance. The composition of the meal and the nature of the excess calories did not affect the changes in $T_0$ FFA. In Study VII there was a marked fall in the elevated $T_0$ FFA when grossly obese subjects were underfed. No such response was observed when two normal weight subjects with low $T_0$ FFA were placed on semi-starvation diets (Study VIII). In all probability, the changes with caloric restriction in the obese group reflected an off-loading of excess preformed tissue FFA. It is also evident from the data in Table 3.9 that the $T_0$ FFA represents a labile pool which is responsive to acute and chronic dietary manipulations. In an attempt to delineate a
mechanism which might control the concentration of tissue FFA and other metabolic parameters and explain the differences in $T_0$ FFA between different subjects, two series of correlation coefficients were calculated. One correlation analysis was derived from data obtained in the absorptive state (Appendix 3) and the other from data obtained in the postabsorptive state (Appendix 3). Data recorded during under- or overfeeding or during prolonged consumption of the eucaloric high carbohydrate diet were not included in these analyses.

(i) Inter-relationships between Adipose Cell Weight and Parameters of Metabolism in Adipose Tissue

(1) In the postabsorptive state. The correlation coefficients listed in Table 3.10 show that significant positive correlations existed between most of the parameters measured. Partial correlation analysis was therefore performed to determine which of the correlations retained their significance when the effects of other variables were taken into account. With this technique the findings were as follows:

(a) lipolysis was a direct function of adipose cell weight independently of the rate of esterification ($r = 0.585; P < 0.01$), FFA release ($r = 0.720; P < 0.001$), change in tissue FFA concentration ($r = 0.726; P < 0.001$) and of the concentration of $T_0$ FFA ($r = 0.744; P < 0.001$);

(b) FFA release was directly proportional to the rate of lipolysis independently of adipose cell weight ($r = 0.759; P < 0.001$), esterification ($r = 0.891; P < 0.001$), $T_0$ FFA concentration ($r = 0.819; P < 0.001$) and the rate of change in tissue FFA ($r = 0.768; P < 0.001$);

(c) the rate of change in tissue FFA (i.e. the rate at which
Table 3.10
Correlations between adipose cell weight and parameters of metabolism in the eucaloric state following a 14 hour fast.*

<table>
<thead>
<tr>
<th></th>
<th>Lipo­lysis</th>
<th>Esteri­fication</th>
<th>FFA Release</th>
<th>Change in Tissue FFA†</th>
<th>Tissue (T₀) FFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose cell weight</td>
<td>0.790 ††</td>
<td>0.767 ††</td>
<td>0.539 †</td>
<td>0.579 †</td>
<td>0.496 †</td>
</tr>
<tr>
<td>Lipolysis</td>
<td></td>
<td>0.664 †</td>
<td>0.817 ††</td>
<td>0.452 †</td>
<td>0.399</td>
</tr>
<tr>
<td>Esterification</td>
<td></td>
<td>0.263</td>
<td>0.732 ††</td>
<td>0.633 †</td>
<td></td>
</tr>
<tr>
<td>FFA release</td>
<td></td>
<td></td>
<td>0.470 †</td>
<td>0.205</td>
<td></td>
</tr>
<tr>
<td>Change in tissue FFA†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.553 †</td>
</tr>
</tbody>
</table>

* Data used for these analyses were the average values obtained in subjects Nos. 1-6, 21-31, n = 17.
† The rate at which tissue FFA concentrations fell during the 2 hour incubation of adipose tissue (T₀-T₂hr).
#/, †, ††, ** Indicate P values < 0.05, < 0.02, < 0.01 and < 0.001 respectively.

tissue FFA concentrations fell during incubation of adipose tissue) increased with increasing rates of esterification independently of adipose cell weight (r = 0.550; P < 0.02), lipolysis (r = 0.648; P < 0.01), T₀ FFA (r = 0.592; P < 0.01) and FFA release (r = 0.714; P < 0.001);

(d) the rate of esterification increased with increasing adipose cell weight independently of the concentration of T₀ FFA (r = 0.674; P < 0.01), the rate of lipolysis (r = 0.528; P < 0.02), FFA release (r = 0.769; P < 0.001) and change in tissue FFA concentration (r = 0.618; P < 0.01);

(e) all other significant correlations listed in Table 3.10 were shown to be spurious. Thus, in the postabsorptive state, FFA release was directly proportional to the rate of lipolysis and the latter was increased in enlarged fat cells. Furthermore,
the rate of change in tissue FFA increased with increasing rates of esterification and esterification was directly proportional to adipose cell weight. However, there was no direct relationship between lipolysis and esterification, each being a separate function of adipose cell weight.

(2) In the absorptive state. These correlation coefficients are listed in Table 3.11 and show fewer significant inter-relationships than those computed in the postabsorptive state. In particular, there was no relationship between the rate of esterification and the rate of change in tissue FFA concentration indicating that the relationship between these parameters recorded in the postabsorptive state did not represent an effect of esterification on tissue FFA. Furthermore, the relationship between esterification and adipose cell weight disappeared when subjects were studied in the absorptive state. Partial correlation analysis of the coefficients listed in Table 3.11 showed that:

(a) lipolysis was a direct function of adipose cell weight independently of the rate of esterification \( (r = 0.793; \ P < 0.001) \), FFA release \( (r = 0.742; \ P < 0.001) \), change in tissue FFA concentration \( (r = 0.826; \ P < 0.001) \) and of the concentration of \( T_0 \) FFA \( (r = 0.821; \ P < 0.001) \);

(b) FFA release was directly proportional to the rate of lipolysis independently of adipose cell weight \( (r = 0.455; \ P < 0.05) \), esterification \( (r = 0.944; \ P < 0.001) \), \( T_0 \) FFA concentration \( (r = 0.645; \ P < 0.01) \) and changes in tissue FFA \( (r = 0.580; \ P < 0.01) \);

(c) all other significant conditions listed in Table 3.11 were shown to be spurious.
Table 3.11

Correlations between adipose cell weight and parameters of metabolism in the eucloric state 1½ hours after a standard high carbohydrate breakfast.*

<table>
<thead>
<tr>
<th></th>
<th>Lipoysis</th>
<th>Esterification</th>
<th>FFA Release</th>
<th>Change in Tissue FFA†</th>
<th>Tissue (T₀) FFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose cell weight</td>
<td>** 0.820</td>
<td>0.363</td>
<td>** 0.522#</td>
<td>-0.190</td>
<td>-0.095</td>
</tr>
<tr>
<td>Lipolysis</td>
<td>0.361</td>
<td>0.650#</td>
<td></td>
<td>-0.395</td>
<td>-0.197</td>
</tr>
<tr>
<td>Esterification</td>
<td></td>
<td></td>
<td>-0.416</td>
<td>0.378</td>
<td>0.464#</td>
</tr>
<tr>
<td>FFA release</td>
<td></td>
<td></td>
<td></td>
<td>-0.426</td>
<td>-0.478#</td>
</tr>
<tr>
<td>Change in tissue FFA†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.518#</td>
</tr>
</tbody>
</table>

* Data used for these analyses were the average values obtained in subjects Nos. 1-4, 7-18, 19a, 19b, 20, n = 19.
† The rate at which tissue FFA concentrations fell during the 2 hour incubation of adipose tissue (T₀-T₂h).
#,** Indicate P values < 0.05, < 0.02, < 0.01 and < 0.001 respectively.

Thus, the data obtained in the absorptive and postabsorptive states indicate that when subjects are in caloric balance, enlarged adipose cells exhibit increased rates of lipolysis and those cells with high rates of lipolysis have high rates of FFA release. One negative finding is of particular interest, viz., the lack of relationship between the rate of FFA uptake or release and the change in concentration of tissue FFA during incubations of adipose tissue. Partial correlation analysis showed the relationship between these parameters in Table 3.10 to be doubtful; and in the absorptive state (Table 3.11), no significant correlation was demonstrated. Furthermore, the dissociation between these parameters is clearly shown by their different responses to the dietary manipulations listed in Tables 3.2, 3.4 and 3.5. In effect, the uptake or release of FFA by adipose tissue and the changes in tissue FFA concentrations in vitro indicate separate metabolic events.
(j) Relationships between Adipose Cell Size, Basal Concentrations of Plasma Glycerol, Insulin, Triglycerides, Cholesterol and FFA

When subjects were in a eucaloric state, there was a positive correlation between adipose cell weight (µg triolein/cell) and the post-absorptive concentration of plasma glycerol \( r = 0.505; \ P < 0.01, n = 24 \), as shown in Figure 3.1. This finding suggests that the observation in vitro (basal lipolysis is a direct function of cell size) in this study and in previous reports [Goldrick and McLoughlin, 1970; Knittle and Ginsberg-Fellner, 1972] was of relevance to the situation in vivo. It also indicates that plasma glycerol concentration is solely derived from lipolytic processes in adipose tissue in vivo [Havel, 1965].

Basal plasma insulin concentration was also proportional to the size of fat cells, as shown by a weak but significant relationship between both metabolic parameters \( r = 0.398; \ P < 0.05, n = 24 \). This observation is in agreement with other reports, which showed the significant positive correlation between fat cell size and plasma insulin level in the basal state or in response to a glucose load [Björntorp et al., 1971a,b; Björntorp and Sjöström, 1971; Stern et al., 1972]. However, this relationship is a negative finding in some studies [Björntorp et al., 1971c; Brook and Lloyd, 1973].

There were no significant relationships between the other plasma constituents (e.g. triglycerides, cholesterol and FFA) and the fat cell size. As well, no significant correlation between the concentrations of metabolites measured in the plasma was demonstrated, except that the basal level of FFA rose with increasing basal triglyceride concentration \( r = 0.469; \ P < 0.02, n = 24 \). The latter result is expected, since in the postabsorptive state, plasma FFA is the major precursor of triglyceride-fatty acids in the plasma VLDL [Barter et al., 1972].
Figure 3.1: Relationship between mean basal plasma glycerol concentration (μmoles/l) and adipose cell size (μg triolein/cell). Data obtained from observation in subjects No. 7-18, 19a, 19b, 20-24 and 26-30) in the eucaloric state. Regression equation:
y = 69.26 x + 13.01; correlation coefficient = 0.505, p < 0.01; n = 24.
3.4 DISCUSSION

In previous studies of the effects of dietary changes on the metabolism of human adipose tissue, two protocols have been used. Some investigators have taken biopsies following an overnight fast [Kjellberg and Östman, 1971; Smith et al., 1973; Salans et al., 1974], in order to eliminate any acute effects on adipose tissue of the preceding meal. This protocol is appropriate for comparing eucaloric and hypocaloric diets. However, it may not be the best approach to comparing eucaloric diets of different composition because any differences observed are not necessarily representative of the situation that exists when a particular diet is being absorbed. Other investigators have attempted to resolve the latter problem by sampling adipose tissue after breakfast [Goldrick and Hirsch, 1964; Sjöström, 1973b]. However, because the breakfast has not been standardized, there is no way of deciding whether the effects of a particular dietary manipulation reflect a change in the composition of the breakfast or a change in composition of the basic diet. The present investigation represents a compromise insofar as the effects on adipose tissue of eucaloric and hypercaloric diets were examined 1½ hours after a standard breakfast. When this mean was given to subjects who had been maintained on the basic control diet used for the present studies, the release of FFA was partly inhibited and there was a small decrease in the concentration of tissue FFA, but lipolysis and esterification were unaffected. The standard breakfast was fed every day, so permitting direct comparisons of eucaloric and hypercaloric dietary changes at lunch and dinner.

The present investigation has shown that isocaloric substitution of carbohydrate for dietary fat had no effect on the metabolism of adipose tissue in vitro observed 1½ hours after the standard breakfast. This is
at variance with results of the previous studies using radioactive glucose [Hirsch and Goldrick, 1964; Salans et al., 1974], which have shown that the rate of glyceride-glycerol synthesis increased by 45-100% during feeding an isocaloric high carbohydrate diet in man. The reason for this discrepancy is probably due to the differences in experimental design.

This work has also demonstrated that the metabolism of adipose tissue in vitro is responsive to changes in caloric balance and that the response to overfeeding depends upon the composition of the diet. Overfeeding with glucose inhibited the release of FFA, reduced the tissue FFA concentrations and stimulated esterification. The net effect of overfeeding with glucose was to cause esterification to exceed lipolysis and so facilitate the uptake of FFA. In contrast, when subjects were overfed with either polyunsaturated or saturated fat, the effects on adipose tissue were quite different. Thus, esterification was unchanged and moderate increases in the rates of lipolysis and FFA release occurred. Nevertheless, the rate of esterification was maintained in excess of lipolysis so that a small net uptake of FFA by adipose tissue was observed during overfeeding with fat. The only similarity between overfeeding with glucose and overfeeding with fat was a decrease in the concentration of tissue FFA. Changes in the metabolism of adipose tissue were also readily demonstrated when obese and non-obese subjects were placed in negative caloric balance. With large caloric deficits, there were marked increases in the rates of lipolysis and FFA release without any change in the rate of esterification. These responses were much greater than those observed during overfeeding with fat, so that lipolysis exceeded esterification by a factor of two with a corresponding increase in the rate of FFA release. The present findings, which
are similar to those reported by Kjellberg and Östman [1971] who also used the balance technique, indicate that fat mobilization is achieved solely by increasing the rate of lipolysis in adipose tissue. They differ from the results of a previous study [Goldrick and Hirsch, 1964] which showed that the incorporation of glucose-$^{14}$C into glyceride-glycerol was depressed during starvation. However, in the latter study, adipose tissue biopsies were taken in the absorptive state prior to caloric reduction. Others using the same protocol as that employed here have failed to show any depression of triglyceride synthesis from palmitate-$^{14}$C during starvation [Kjellberg and Östman, 1971].

Some idea can be obtained of the accuracy with which in vitro rates of glycerol and FFA production in adipose tissue reflect the situation in vivo, by comparing the adipose tissue data with the dietary-induced changes in basal plasma glycerol and FFA concentrations. Overfeeding with glucose caused no change in the plasma glycerol, but lowered the plasma FFA indicating an increased rate of esterification in adipose tissue in the postabsorptive state. Similar changes were recorded in adipose tissue 1½ hours after the standard breakfast. Overfeeding with fat led to a small increase in the plasma glycerol concentration; and a small increase in the rate of glycerol production by adipose tissue was observed. Net FFA production in vitro and plasma FFA concentrations were unaffected. Furthermore, with semi-starvation, the plasma glycerol and FFA concentrations were increased and glycerol and FFA production in adipose tissue were markedly stimulated. On the other hand, the eucaloric high carbohydrate diet had no effect on lipolysis or esterification in adipose tissue and failed to induce any change in the concentrations of plasma glycerol or FFA. It is clear that the rates of lipolysis and esterification in adipose tissue were fairly
representative of those existing in vivo in the postabsorptive state. The fact that such a relationship was demonstrable in the eucaloric and hypercaloric dietary studies implies that either the standard breakfast failed to evoke a significant response in adipose tissue or if a response did occur it was largely determined by the metabolic status of adipose tissue prior to the meal.

Measurements of plasma cholesterol and triglycerides were carried out primarily to monitor the effects of the various dietary manipulations. The responses of these plasma lipids to changes in dietary fat and carbohydrate have been thoroughly investigated [Ahrens et al., 1957; Farquhar et al., 1966; Nestel, 1973; Nestel et al., 1974; Schonfeldt et al., 1976]; and the changes in the concentrations of plasma cholesterol and triglycerides observed here indicated that each of the diets achieved its desired effect. Glycerol and FFA are not the sole precursors of plasma triglycerides [Nestel, 1973] and dietary exchanges produce complex changes in the composition and concentration of plasma lipoproteins [Schonfeldt et al., 1976]. Therefore, there was no clear relationship between lipolysis and esterification in adipose tissue and the concentrations of plasma cholesterol and triglycerides, as shown in this study. Overfeeding with polyunsaturated fat lowered the plasma cholesterol and triglycerides whereas overfeeding with saturated fat had no such effect. However, the response of adipose tissue to overfeeding was the same with both types of fat. Likewise, the eucaloric high carbohydrate diet and overfeeding with glucose both raised the plasma triglycerides and lowered the plasma cholesterol, but the responses of adipose tissue to the two diets were different.

It is probable that the responses of adipose tissue to overfeeding with glucose and overfeeding with fat reflected different states of
insulin secretion and insulin sensitivity. Isocaloric high carbohydrate diets, fed to man, are known to enhance the effects of insulin on glucose metabolism in adipose tissue in vitro [Salans et al., 1974] and increase insulin sensitivity in vivo [Himsworth, 1935]. In this study, overfeeding with glucose caused a small increase in the concentration of plasma insulin; and this combined with an enhanced state of insulin sensitivity could well account for the acceleration of esterification observed in adipose tissue [Goldrick, 1967a; Lisch et al., 1973]. High fat diets on the other hand elevate the fasting blood glucose, cause glucose intolerance and insulin resistance in vivo and impair the antilipolytic effects of insulin on adipose tissue in vitro when fed to rats [Ogundipe and Bray, 1974; Smith et al., 1974]. Insulin resistance and impaired glucose tolerance also occur in man if fat is substituted for dietary carbohydrate [Himsworth, 1935]. When subjects participating in this study were overfed with fat there was no change in the basal plasma insulin, but lipolysis in adipose tissue increased, as might be expected if the antilipolytic effects of insulin were impaired.

It is clear that an increased rate of esterification in adipose tissue in the postabsorptive state and early in the morning is not an inevitable accompaniment of fat deposition in man. If this situation exists throughout a 24 hour period during overfeeding, then presumably a small net uptake of FFA is sufficient for adipose tissue to clear triglyceride-fatty acids from the plasma. On the other hand, it is possible that the uptake of triglyceride-fatty acids by adipose tissue is a cyclical phenomenon and occurs late in the day. Certainly, diurnal fluctuations in plasma insulin, FFA and triglycerides occur during the feeding of high carbohydrate diets [Barter et al., 1971], but there is no comparable information on the effects of overfeeding with glucose or fat.
When adipose tissue is obtained in the postabsorptive state, the rates of glycerol production and triglyceride synthesis from glucose-1^4C are directly proportional to fat cell size [Goldrick and McLoughlin, 1970; Björntorp and Karlsson, 1970; Knittle and Ginsberg-Fellner, 1972]. Such increased rates of triglyceride turnover in enlarged fat cells may well be responsible for the high rates of turnover of plasma FFA in obese individuals [Nestel and Whyte, 1968]. The basis for these metabolic changes in enlarged fat cells has not been defined. However, it has been suggested that in rat adipose tissue, high rates of lipolysis stimulate esterification by elevating the intracellular concentration of FFA [DiGirolamo et al., 1974]. Partial correlation analyses of data obtained in the eucaloric state in this study have shown that the rate of FFA release from adipose tissue is directly proportional to the rate of lipolysis and that the latter is increased in enlarged fat cells irrespective of whether the tissue is obtained in the postabsorptive state or following a high carbohydrate meal. The rate of esterification of FFA was also proportional to fat cell size in the postabsorptive state. However, this relationship was independent of the rate of lipolysis, tissue FFA concentrations and other parameters measured when adipose tissue was obtained in the postabsorptive state; and was no longer demonstrable when adipose tissue was sampled after the high carbohydrate meal. Evidently there is a very close relationship between adipose cell size and the rate of lipolysis and between lipolysis and FFA release, but it is difficult to see how the rate of lipolysis or the concentration of tissue FFA has any bearing on the rate of esterification.

Correlation analysis also showed that the rate of release of FFA was independent of the concentration of tissue FFA and of the changes in...
tissue FFA concentrations that occurred during incubation of adipose tissue. This dissociation of events was also clearly demonstrated by the feeding of different diets. The tissue FFA concentrations almost invariably declined when adipose tissue was incubated, but the rate of change was not influenced by the standard high carbohydrate meal or any of the hypercaloric diets. The concentrations of tissue FFA at the commencement of incubations of adipose tissue were reduced by the standard meal and by all the hypercaloric diets. On the other hand, the direction and rate of movement of FFA between adipose tissue and the incubation medium responded in a specific way to the various dietary manipulations. These findings are incompatible with the proposition that FFA diffuse freely across the membrane of the fat cell, and that the direction and rate of flux are solely dependent on the concentration gradient of FFA between the cytoplasm and the extracellular fluid [Vaughan, 1962]. They are however consistent with other observations in rat adipose tissue. The latter have shown that FFA which arise from the breakdown of triglycerides and those that are taken up by the fat cell occupy separate pools [Dole, 1961; Vaughan et al., 1964]. The presence of different pools of tissue FFA implies separate mechanisms for FFA uptake and release. In the latter connection it should be noted that an energy dependent process for the release of FFA has been described and this is under separate metabolic control from that of lipolysis [Schimmel and Goodman, 1971]. It remains to be seen whether another system exists for transporting FFA from the extra-cellular fluid into the cytoplasmic compartment.

As has already been discussed, the rate of lipolysis in adipose tissue in vitro is increased in direct proportion to increasing cell size. This relationship was observed in tissue obtained both in the
postabsorptive state and after taking the high carbohydrate meal. Lipolysis in adipose tissue *in vivo* in the basal state, indirectly estimated as the basal plasma glycerol concentration, presumably is a direct function of fat cell size, since the postabsorptive level of plasma glycerol was directly proportional to the size of the fat cell. This finding supports an increased rate of lipolysis in enlarged fat cells *in vivo*. The latter phenomenon is partly responsible for the cause of increased triglyceride turnover that was observed in human obesity [Nestel and Whyte, 1968; Björntorp et al., 1969a; Bortz et al., 1972]. In the present study, a weak but positive correlation between adipose cell size and basal plasma insulin was also found. This is in agreement with previous reports (Björntorp et al., 1971b; Stern et al., 1972]. This finding only indicates that hyperinsulinaemia is commonly associated with adipose cell enlargement. However, any metabolic relationship between these two parameters can not be explained in this study.

### 3.5 SUMMARY

The effects of dietary manipulations on lipolysis and esterification in human adipose tissue were examined by measuring the production of glycerol and FFA *in vitro* on biopsy samples of subcutaneous fat. Serial biopsies were performed on subjects before and during consuming experimental diets, 1½ hours after a standard high carbohydrate breakfast (P:F:C = 8:25:67%). Postabsorptive concentrations of plasma glucose, insulin, cholesterol, triglycerides, FFA and glycerol were also serially determined. Feeding a eucaloric high carbohydrate diet (P:F:C = 13:13:74%, 4-6 days) elevated the plasma triglycerides, but had no effect on lipolysis or esterification in adipose tissue. Feeding a hypercaloric high carbohydrate diet (the eucaloric high
carbohydrate diet plus an additional 30% of calories as glucose, 
P:F:C = 11:10:79\% for 4-9 days caused increases in the basal plasma, 
triglyceride and insulin levels. The plasma FFA and cholesterol levels fell. Esterification in adipose tissue \textit{in vitro} increased by 36\% to exceed the rate of lipolysis. The net uptake of FFA by adipose tissue was increased, reflecting an inhibition of FFA release. On the other hand, 
overfeeding with saturated fat (a eucaloric standard diet plus 50\% of 
calories as saturated fat, P:F:C = 11:56:33\%) for 8-12 days did not cause 
hypertriglyceridaemia and had no effect on esterification but stimulated 
lipolysis by 29\%. The rate of esterification exceeded the rate of lipo-
ysis, maintaining the net uptake of FFA in the incubation system. The 
postabsorptive plasma glycerol concentration rose during overfeeding 
with saturated fat. When the eucaloric control diet was supplemented 
with an additional 50\% of calories from polyunsaturated fat 
(P:F:C = 11:57:32\%) for 15 days, the plasma cholesterol and triglyceride concentrations fell but plasma glycerol did not alter. The plasma 
glucose was increased. In other respects, the effects of overfeeding 
polyunsaturated fat were similar to those of overfeeding with saturated 
fat.

When grossly obese subjects were placed in negative caloric 
balance (a reduction of 3,150 calories per day), the concentrations of 
plasma cholesterol, triglycerides and insulin fell and the plasma 
glycerol and FFA rose. There was no change in the rate of esterifica-
tion in adipose tissue. Lipolysis was increased by 74\%, FFA release by 
134\% and the rate at which tissue FFA decreased \textit{in vitro} (during incubation of 
adipose tissue) fell to one third of the values in the eucaloric state. 
Two non-obese subjects responded to deficits of 1,900-2,400 calories per 
day with elevated plasma FFA and reduced plasma glucose concentrations.
Lipolysis in adipose tissue was increased by 68%, esterification was unaltered and the net production of FFA (tissue plus medium) was increased by 136%.

Data obtained in the eucaloric state as well as the responses of adipose tissue to dietary manipulations showed that the uptake and release of FFA from adipose tissue were independent of changes in tissue FFA concentration.

Tissue FFA concentrations ($T_0$ FFA) measured after a 30 minute preincubation were diminished during overfeeding with high carbohydrates or high fat diets. Grossly obese subjects submitted to starvation had a reduction in $T_0$ FFA, which was not found in adipose tissue of fasting lean subjects.

The inter-relationships between adipose cell weight and other parameters of metabolism in adipose tissue in vivo during feeding an isocaloric control diet to subjects were analysed. Enlarged fat cells exhibited increased rates of lipolysis, the latter parameter being directly proportional to the rate of FFA release. They were observed in the absorptive and postabsorptive states. There was no significant correlation between the concentration of tissue FFA ($T_0$) and the rate of change in tissue FFA concentration during incubation of adipose tissue or between either of both parameters and FFA release. Moreover, no direct relationship between lipolysis and esterification rate was demonstrated, indicating that each parameter was a separate function of fat cell size.
Conclusions

(1) lipolysis and esterification in human adipose tissue are responsive to changes in caloric balance;

(2) the response to overfeeding depends on the composition of the diet;

(3) changes in lipolysis and esterification in adipose tissue parallel dietary induced changes in basal plasma FFA and glycerol but not changes in plasma cholesterol or triglycerides;

(4) factors that control the uptake and release of FFA by adipose tissue differ from those that govern the concentration of tissue FFA;

(5) lipolysis and esterification are separate functions of adipose cell size.
CHAPTER 4

EFFECTS OF OVERFEEDING ON CARBOHYDRATE TOLERANCE, INSULIN SECRETION, ESTERIFICATION AND LIPOLYSIS IN MAN

4.1 INTRODUCTION

In 1935, Himsworth showed that isocaloric substitution of carbohydrate for dietary fat improved glucose tolerance and increased sensitivity to exogenous insulin in man. Administration of a low carbohydrate-high fat diet had the converse effect, the deterioration in glucose tolerance and insulin sensitivity being proportional to the low content of dietary carbohydrate [Himsworth, 1935]. More recent studies have confirmed these findings [Wales et al., 1967; Brunzell et al., 1971; Anderson et al., 1973]. They have also shown that sensitivity to endogenous insulin is increased when isocaloric high carbohydrate diets are fed to normal subjects [Brunzell et al., 1971; Anderson et al., 1973]. On the other hand, overfeeding with a basic diet in man causes increased insulin response to a glucose load without impaired carbohydrate tolerance [Olefsky et al., 1975]. There is no information on the effects of overfeeding with fat on carbohydrate tolerance and insulin secretion in normal subjects. In the present investigation, carbohydrate tolerance and plasma insulin levels were measured in healthy young adults before and during overfeeding with high carbohydrate or high fat diets. The effects of overfeeding on lipolysis and esterification in adipose tissue were assessed indirectly by measuring
the concentrations of plasma glycerol and free fatty acids before and after a standard high carbohydrate breakfast when the subjects were in caloric balance and when they were overfed.

4.2 MATERIALS AND METHODS

Subjects comprised eleven healthy young volunteers. Their clinical details are summarized in Table 4.1. Other details of experimental condition are described in Chapter 2 (Materials and Methods).

Table 4.1
Clinical Data

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* Ideal body weights were computed from tables of standard body weights [Society of Actuaries, 1959].
Experimental Design

Dietary manipulations for the subjects have been outlined in Chapter 2, Section 2.1, and details of dietary composition for individual subjects are given in Appendix 2. In brief, all subjects were maintained for 6-10 days on a standard eucaloric diet (P:F:C = 15:39:46%). The mean daily cholesterol intake was 466 mg. A standard high carbohydrate breakfast containing 750 calories (P:F:C = 8:25:67%) was fed daily to each subject whilst consuming standard and hypercaloric diets. The composition and quantity of calories given at lunch and dinner were adjusted to give the required daily intake of calories, protein, fat and carbohydrate.

At the end of the control period, subjects were overfed either a hypercaloric high carbohydrate or high fat diet for 14 days. Subjects Nos. 1-4 were placed on a eucaloric high carbohydrate diet plus an additional 30% of calories as glucose (P:F:C = 11:9:80%). The daily cholesterol intake averaged 154 mg. Subjects Nos. 5-7 were maintained on the standard eucaloric diet which was supplemented with an additional 30% of calories predominantly as saturated fat (P:F:C = 12:55:33%). The p/s ratio of the latter diet was 0.11, and daily cholesterol intake was 797 mg. Subjects Nos. 8-11 were maintained on the standard eucaloric diet which was supplemented with an additional 50% of total calories as saturated fat (P:F:C = 11:59:30%). This hypercaloric high fat diet had a p/s ratio of 0.07, and 1009 mg of cholesterol per day.

On days 2, 4, 7 of the eucaloric diet and on days 4, 7, 11, 14 of each of the hypercaloric diets, blood samples were taken after a 12 hour overnight fast (through a 19-gauge scalp vein needle). The needle was prevented from occlusion by a slow infusion of normal saline solution.
The standard high carbohydrate breakfast was taken over a 20 minute period. Blood samples were then withdrawn at half hourly intervals after the meal over a 2 hour period. They were used for determinations of blood glucose and plasma insulin, glycerol and free fatty acids (FFA). The subjects remained recumbent throughout the carbohydrate tolerance test. Blood glucose concentrations were measured on the experimental day. Other procedures for collection and measurement of blood specimens as well as statistical analyses are also described in Chapter 2. Data are presented as means ± 1 S.E.M.

4.3 RESULTS

(a) Effects of Supplementing the Eucaloric High Carbohydrate Diet with Glucose

Body weights rose from a mean value of 57.4 kg on the standard eucaloric diet to 59.6 kg (P < 0.001) on the last day of the hypercaloric high carbohydrate diet. As shown in Figure 4.1, the hypercaloric high carbohydrate diet had no effect on the basal plasma insulin concentration or on the insulin response to the high carbohydrate breakfast. The basal blood glucose concentration was also unaffected by overfeeding. However, carbohydrate tolerance was enhanced as shown by lower blood glucose concentrations at ½, 1 and 1½ hours after the standard breakfast. The latter changes were observed on day 4 of overfeeding and there was no evidence to indicate that any further improvement in carbohydrate tolerance occurred later in the study. The basal plasma glycerol concentration and the values obtained after ingestion of the high carbohydrate breakfast were similar during both dietary regimes. On the other hand, the basal plasma FFA were significantly reduced when the hypercaloric high carbohydrate diet was fed and this reduction was
Figure 4.1: Blood glucose, plasma concentrations of insulin, glycerol and FFA before and after ingestion of the high carbohydrate breakfast in subjects 1-4 maintained on the standard eucaloric diet (○—○) and on the eucaloric high carbohydrate diet supplemented with an additional 30% of total calories as glucose (□—□). Values are given as means ±1 S.E.M.

*, **, *** indicate p values < 0.05, < 0.01, < 0.001 respectively. These were obtained by comparing data recorded during feeding standard eucaloric and hypercaloric high carbohydrate diets.
generally maintained after ingestion of the standard breakfast. The changes in plasma FFA occurred by day 4 of overfeeding and no further reductions in plasma FFA were observed later in the study. These findings indicate that glucose utilization in response to insulin was enhanced during overfeeding with the high carbohydrate diet. Both the basal and absorptive rates of re-esterification of fatty acids in adipose tissue appeared to be increased by this dietary manipulation, whereas the glycerol data suggest that lipolysis in adipose tissue was unaffected.

(b) Effects of Supplementing the Standard Eucaloric Diet with Saturated Fat

Body weights rose from a mean value of 62.1 kg on the standard eucaloric diet to 63.6 kg ($P < 0.05$) on the last day of the study when subjects Nos. 5-7 received an additional 30% of calories as saturated fat. In subjects Nos. 8-11 who received an additional 50% of calories as saturated fat, their body weights rose from 71.1 kg to 74.0 kg ($P < 0.001$) on the last day of the study.

Since the metabolic responses of subjects Nos. 5-11 to each level of overfeeding were similar, the data have been pooled and are summarized in Figure 4.2. The hypercaloric high fat diet had no effect on the basal plasma insulin concentration or on the insulin response to the standard breakfast. In this respect, the effects of overfeeding with fat were similar to overfeeding with carbohydrate. On the other hand, not only were the postabsorptive concentrations of blood glucose elevated during overfeeding with fat, but also carbohydrate tolerance was slightly but significantly reduced at 1½ and 2 hours after the standard high carbohydrate breakfast. These changes in blood glucose
Figure 4.2: Blood glucose, plasma concentrations of insulin, glycerol and FFA before and after ingestion of the high carbohydrate breakfast in subjects 5-11 maintained on the standard eucaloric diet (closed circle) and on the standard eucaloric diet supplemented with an additional 30% or 50% of total calories as saturated fat (open circle). Values are given as means ± S.E.M.

*, **, *** indicate p values < 0.05, < 0.01, < 0.001 respectively. These were obtained by comparing data recorded during feeding of standard eucaloric and hypercaloric high fat diets.
concentration were observed on day 4 of overfeeding and no further changes occurred later in the course of administering the hypercaloric high fat diets. The latter, like the hypercaloric high carbohydrate diet, depressed the postabsorptive concentrations of plasma FFA and these lower concentrations were maintained after ingestion of the standard breakfast. It therefore appears that overfeeding increases the rate of re-esterification of fatty acids in adipose tissue; and this effect is independent of the composition of the diet. When subjects Nos. 5-11 were overfed with fat the postabsorptive concentrations of plasma glycerol were unaltered. However, after ingesting the standard breakfast the plasma glycerol actually rose and elevated glycerol concentrations were maintained for 2 hours. This response of the plasma glycerol to the standard high carbohydrate breakfast was a regular finding throughout the entire period of overfeeding with fat. Evidently, when healthy young subjects are overfed with fat, the effects of insulin on the disposal of glucose are slightly impaired, and the antilipolytic effects of insulin on adipose tissue are markedly attenuated.

4.4 DISCUSSION

The present investigation has shown that overfeeding with a high carbohydrate diet enhanced carbohydrate tolerance whilst overfeeding with fat had the reverse effect. Neither overfeeding regime altered the insulin response to a high carbohydrate meal. In these respects, the present findings are similar to those of previous studies in humans which have shown improved glucose tolerance and increased sensitivity to exogenous and endogenous insulin when carbohydrate is substituted.
isocalorically for dietary fat, and the reverse effects when fat is substituted for dietary carbohydrate [Himsworth, 1935; Hales and Randle, 1963a; Brunzell et al., 1971; Anderson et al., 1973]. In all probability these dietary effects are mediated via the liver and represent variations in the extraction of glucose from the portal circulation in response to endogenous insulin. The liver utilizes up to 60% of an orally administered glucose load [Felig et al., 1975] and is highly sensitive to small changes in plasma insulin [Felig and Wahren, 1971]. Additionally, hepatic glucokinase activity in man is induced by dietary carbohydrate [Borrebaek et al., 1970] indicating an increased capacity to utilize glucose. Conversely, the fat-low carbohydrate diets reduce the activity of glucokinase in man [Borrebaek et al., 1970], stimulate gluconeogenesis in rats [Eisenstein et al., 1974] and presumably depress hepatic glucose uptake. It appears that human adipose tissue has a low capacity to utilize glucose [Björntorp et al., 1971a; Sjöström, 1973b], even though basal glucose metabolism and insulin sensitivity may be enhanced by high carbohydrate diets [Hirsch and Goldrick, 1964; Salans et al., 1974]. Furthermore, forearm metabolic studies have failed to demonstrate any adaptive change in human muscle in response to an infusion of insulin following exchanges of isocaloric high and low carbohydrate diets [Horton et al., 1972].

Isocaloric substitution of carbohydrate for dietary fat suppresses the plasma FFA concentrations in the postabsorptive state and following an oral glucose tolerance test [Hales and Randle, 1963a; Wales et al., 1967] suggesting that dietary carbohydrate either depresses lipolysis or stimulates esterification in adipose tissue. During overfeeding a similar situation might well be anticipated since a mechanism should exist for storing excess triglyceride-fatty acids in adipose tissue in
states of positive caloric balance. In the present investigation these predictions were only partly borne out. In subjects overfed with glucose or saturated fat, the postabsorptive concentrations of plasma FFA were suppressed as were the plasma FFA following the standard high carbohydrate meal; and these changes were independent of the composition of the diet. Overfeeding with a high carbohydrate diet had no effect on the postabsorptive or absorptive concentrations of plasma glycerol, indicating that lipolysis in adipose tissue was unaltered in the face of increased rate of esterification. In this situation the storage of triglyceride-fatty acids should be enhanced. Similarly, overfeeding with fat caused no change in basal lipolysis as judged from the postabsorptive concentrations of plasma glycerol, but the plasma glycerol concentrations actually rose after ingestion of the high carbohydrate meal when the subjects were overfed with fat. Studies in vitro with rat adipose tissue have shown that high fat diets impair the anti-lipolytic effects of insulin on stimulated lipolysis [Smith et al., 1974], without affecting basal lipolysis [Ogundipe and Bray, 1974]. The present findings suggest that a similar phenomenon may occur in man. To what extent the changes in lipolysis modify the storage of dietary triglyceride-fatty acids during overfeeding with fat remains to be seen.

4.5 SUMMARY

Blood glucose, plasma concentrations of insulin, free fatty acids (FFA) and glycerol were measured in 11 healthy lean young adults, in response to a standard high carbohydrate meal, before and during overfeeding with a high carbohydrate or a high fat diet. The plasma insulin was unaffected by overfeeding, whilst the hypercaloric high carbohydrate
diet enhanced carbohydrate tolerance and the hypercaloric high fat diet had the reverse effect. Both types of overfeeding depressed the post-absorptive and absorptive concentrations of plasma FFA. Overfeeding with the high carbohydrate diet had no effect on the plasma glycerol concentrations recorded before and after ingestion of the standard breakfast. Overfeeding with high fat diet also failed to alter the postabsorptive concentration of plasma glycerol but the plasma glycerol levels rose following the standard high carbohydrate meal. These findings indicate that:

(a) overfeeding with a high carbohydrate diet enhances the utilization of glucose by increasing sensitivity to insulin, whilst overfeeding with a high fat diet has the opposite effect;

(b) esterification of FFA in adipose tissue is stimulated by overfeeding independently of the composition of the diet;

(c) basal lipolysis in adipose tissue (as judged by the post-absorptive plasma glycerol concentrations) is unaffected by overfeeding but the antilipolytic effects of insulin on adipose tissue are impaired following the standard meal, when subjects are overfed with a high fat diet.
CHAPTER 5
GENERAL DISCUSSION

Deposition and mobilization of fat in adipose tissue are regulated by hormonal and nutritional factors. In studies with human adipose in vitro, insulin (at physiological concentrations) has been shown to promote the utilization of glucose for oxidation and esterification of FFA [Björntorp, 1967a; Owen et al., 1967; Goldrick, 1967a; Salans et al., 1974]. Insulin also exerts its antilipolytic effect on stimulated lipolysis [Burns and Langley, 1968; Moskowitz and Pain, 1969; Goldrick and McLoughlin, 1970; Lisch et al., 1974]. Nutritional states of fat donors also modify glucose metabolism by adipose tissue in the basal conditions and in the presence of insulin in vitro. A restricted calorie diet gives rise to inhibition of the stimulating effect of insulin on glucose metabolism [Bray, 1969; Davidson, 1975]. A eucaloric high fat-low carbohydrate diet causes decreases in basal and insulin stimulated glucose oxidation [Sims et al., 1973]. In contrast, isocaloric substitution of carbohydrate for dietary fat leads to increases in basal and insulin augmented-glucose metabolism in vitro [Hirsch and Goldrick, 1964; Salans et al., 1974]. It has been assumed that changes in adipose tissue metabolism observed in vitro are partly representative of the metabolic and hormonal status occurring in vivo before sampling of adipose tissue [Fessler et al., 1967]. At present, there is no information to indicate the contributions of both the rates
of lipolysis and esterification in regulating the deposition and mobilization of fat in human adipose tissue in response to variations in substrate intake of the tissue. Likewise, it remains unclear whether changes in metabolic activities of adipose tissue are induced by the dietary factors (i.e. composition and caloric balance) themselves or are mediated by the effects of endogenous insulin. On the other hand, a precise cause of insulin resistance commonly associated with human obesity can not be provided [see a review by Nestel and Goldrick, 1976]. Dietary factors appear to have more influence than adipose cell size on modifying the effect of insulin in vitro on glucose metabolism in human adipose tissue [Salans et al., 1974].

In the present investigation, the contributions of lipolysis and esterification in the regulation of fat storage and breakdown in human adipose tissue were investigated. The intake of substrates into adipose tissue in vivo was varied by giving diets of differing composition or caloric content to the subjects for 1-2 weeks. The rates of lipolysis and esterification, as well as other metabolic parameters (FFA release, change in tissue FFA, net change in FFA in the incubation system), were measured serially in adipose tissue biopsy samples in vitro during consumption of a eucaloric standard diet (P:F:C = 15:39:46%) and experimental diets. The effects on adipose tissue of changes in dietary composition or caloric intake were examined 1½ hours after a standard high carbohydrate breakfast (P:F:C = 8:25:67%, 750 calories). A eucaloric high carbohydrate diet (P:F:C = 12:16:72%) was given in 3 equal portions (at breakfast, lunch and dinner) to obese subjects on an isocaloric state. Data of adipose tissue metabolism in vitro observed following the meals were pooled and compared with those recorded following an overnight fast. There were no changes in the rates of
lipolysis and esterification. The FFA release into the medium, net change in FFA in the assay system and tissue FFA concentration were slightly but significantly decreased, during the absorptive state. These data indicate that the rates of lipolysis and esterification in adipose tissue in vitro, under the experimental conditions used in this study, were unaffected by an acute carbohydrate load. This finding is similar to the observation of Mellati et al. [1970], who showed that a standard mixed meal had no effect on the incorporation of labelled glucose into CO₂ and glyceride-glycerol in adipose tissue observed 1-1½ hours after the meal, but stimulated glyceride-fatty acid synthesis from glucose. In their study, normal healthy subjects were maintained on a standard high caloric diet consisting of 300 g of carbohydrate for 4 days, and the mixed meal contained one-third of the daily caloric and carbohydrate intake. Their subjects also responded to the test meal by showing a rise in the serum insulin and a fall in the plasma FFA concentration [Mellati et al., 1970], indicating increased esterification or decreased lipolysis in adipose tissue in vivo. A definite reason for the different results obtained from in vitro and in vivo studies can not be given. However, it should be noted that the metabolism of adipose tissue in vivo is regulated by the net effect of several hormones and metabolic processes in other tissues. It is probable that some metabolic changes occurring in vivo cannot be detected when adipose tissue is studied in an artificial environment.

The effect of an acute load of substrates on metabolism of adipose tissue in vitro was not the aim of this study. Data obtained were used only to consider to what extent the standard meal might affect adipose tissue metabolism. Therefore, changes in the plasma constituents following the meals were not measured. When it appeared that the high
carbohydrate meal caused only minor changes in the metabolic activity of adipose tissue, the standard high carbohydrate breakfast (of similar composition to the high carbohydrate meals) was used as a constant stimulus in the comparison of the effects of eucaloric diets of different composition or the effects of eucaloric and hypercaloric diets, given at lunch and dinner. An acute effect of a eucaloric high fat meal was also examined in adipose tissue of obese subjects. The latter meal produced lesser changes in adipose tissue metabolism than did the high carbohydrate meal, only a diminution in tissue FFA concentration was observed in the absorptive state. However, the high carbohydrate meal was chosen, since it was better tolerated.

In response to the standard high carbohydrate breakfast, parameters of adipose tissue metabolism observed in vitro were not altered by increasing the carbohydrate content of the eucaloric diet (P:F:C = 13:13:74%). This was in spite of a significant elevation of the basal plasma triglyceride levels. Feeding a eucaloric high carbohydrate diet to subjects was expected to produce some changes in adipose tissue metabolism. Moreover, the result recorded in adipose tissue in vitro in this study is at variance with other reports [Hirsch and Goldrick, 1964; Salans et al., 1974], which showed that a eucaloric high carbohydrate diet increased the transformation of labelled glucose to adipose tissue triglycerides. The cause of the contradictory result may be explained in part by the different experimental design. In the study of Hirsch and Goldrick [1964], a high carbohydrate-fat free formula diet (P:F:C = 15:0:85%) was given to the subjects for 1 month, and adipose tissue biopsies were performed 2½ hours after having breakfast. The latter meal was not standardized, and the observed changes in adipose tissue may represent the responses to a difference in composition of the
meal before adipose tissue sampling, or to a change in composition of the basic diet taken during the day. Salans et al. [1974] studied the effect of a eucaloric high carbohydrate diet (P:F:C = 15:15:70%), which consisted of a percentage of calories derived from carbohydrate similar to that contained in this study (P:F:C = 13:13:74%). However, in their studies, adipose tissue biopsies were performed in the postabsorptive state, and the changes in adipose tissue metabolism induced by the high carbohydrate eucaloric diet were compared with data obtained during feeding the low carbohydrate eucaloric diet.

On the other hand, it should be noted that the eucaloric high carbohydrate diet was given to 8 subjects, some of whom were grossly obese (Nos. 7, 11, and 12), and some were mildly hypertriglyceridaemic (Nos. 9, 12, 14). The metabolic responses in adipose tissue to this diet were similar in these subjects so the data were pooled for analysis. However, the inherent metabolic abnormalities in obese subjects [Sims et al., 1973] and hypertriglyceridaemic subjects [Larsson et al., 1975], may account for the lack of adipose tissue response to the eucaloric high carbohydrate diet in the present study. Moreover, it has been demonstrated that adipocytes obtained from endogenous hypertriglyceridaemic subjects are enlarged, and exhibit increases in hexokinase isoenzyme activities and incorporation of labelled glucose into triglycerides [Bernstein et al., 1975]. Additionally, enlarged adipocytes of hypertriglyceridaemic subjects have an increased basal and norepinephrine-stimulated lipolysis, but the antilipolytic effect of insulin on the stimulated lipolysis is decreased [Larsson et al., 1975]. Therefore, if all subjects participating in this dietary regimen were lean and healthy, the response of adipose tissue may have been different.

Data of parameters of adipose tissue metabolism recorded in the
absorptive state were assumed to be representative of metabolic events existing \textit{in vivo} in the postabsorptive state. Therefore, changes in the postabsorptive concentrations of metabolites in the plasma (glucose, insulin, FFA, glycerol) were compared with changes in the adipose tissue metabolism. The metabolic activities observed in adipose tissue during consumption of the eucaloric high carbohydrate diet were consistent with the metabolic processes occurring \textit{in vivo}, since there were no changes in the concentrations of plasma glucose, insulin, FFA and glycerol in the postabsorptive state. However, some questions still arise: whether adipose tissue metabolism in the absorptive state is mainly governed by the metabolic events prior to the meal, or the response of adipose tissue following the meal is altered by the eucaloric high carbohydrate diet but the experimental conditions \textit{in vitro} is inappropriate to detect any change.

In the present study, overfeeding with glucose or fat led to a small but significant increase in body weight. This presumably indicates an increase in body fat mass, as shown in other reports [Sims \textit{et al.}, 1968; Sims \textit{et al.}, 1973]. Moreover, this study has shown that changes in the state of caloric balance, i.e. hypercaloric or hypocaloric administration, modify metabolic activities of adipose tissue \textit{in vitro}. Changes in adipose tissue metabolism \textit{in vitro} induced by overfeeding with carbohydrate (recorded 1.5 hours after the standard meal) were appropriate with an increased utilization of glucose for the esterification of FFA (to be stored as triglycerides). The increased rate of esterification, as estimated by the balance method, was similar to the finding of Sjöström [1973b], who measured the incorporation of glucose-$^{14}$C into adipose tissue triglycerides. Moreover, overfeeding with glucose inhibited the release of FFA and facilitated the uptake of
FFA by adipose tissue. This observation suggests that increases in the rates of FFA uptake and esterification in adipose tissue may be regulatory mechanisms for clearing excess triglyceride fatty acids from the plasma. It has been suggested that the removal of plasma triglycerides by adipose tissue is determined not only by lipoprotein lipase (LPL) activity but also by a mechanism for removal of FFA liberated by the enzyme [Walldius et al., 1974; Carlson and Walldius, 1976]. The rate of uptake or esterification of exogenous fatty acids in adipose tissue from hypertriglyceridaemic subjects in the postabsorptive state was lower than in normal subjects [Carlson and Walldius, 1976]. In contrast, this finding has not been confirmed in another study in which diabetic subjects were excluded [Clifton-Bligh and Galton, 1976]. The present work has shown that overfeeding with glucose in man causes an elevation of basal plasma triglyceride concentration, enhances FFA uptake by adipose tissue in vitro, recorded in the absorptive state (after breakfast). However, whether this adaptive mechanism consistently occurs during the day remains speculative. Likewise, the extent of contribution of increases in uptake and esterification of FFA in adipose tissue to the clearance of plasma triglyceride fatty acids requires further investigation.

The effects of overfeeding with either saturated or polyunsaturated fat on adipose tissue metabolism in vitro were quite different from overfeeding with carbohydrate. The rate of esterification and net change in FFA in the incubation system were unaltered. Conversely, the rate of lipolysis in the absorptive state was moderately increased. However, the rate of esterification exceeded the rate of lipolysis resulting in a small net uptake of FFA by adipose tissue during overfeeding with fat. In previous reports, an isocaloric low carbohydrate
high fat diet given to man led to a decreased oxidation of glucose by adipose tissue [Sims et al., 1973; Salans et al., 1974]. Adipose tissue from rats fed a high fat diet exhibited diminution of glucose oxidation and inhibition of lipogenesis from glucose, acetate and pyruvate [Hausberger and Milstein, 1955; Zaragoza-Hermans and Felber, 1972; Lavau and Susini, 1975]. On the other hand, a high fat diet fed to rats caused no change in the rate of incorporation of labelled glucose into adipose tissue glyceride-glycerol \textit{in vitro} [Smith et al., 1974] or caused an increased rate of glucose incorporated into triglycerides \textit{in vivo} [Lavau and Susini, 1975].

During overfeeding with carbohydrate or fat, the responses of adipose tissue in the absorptive state (observed \textit{in vitro}) may partly reflect the situation existing \textit{in vivo} in the postabsorptive state, since the changes in basal plasma glycerol and FFA concentrations induced by these dietary manipulations were comparable with the data obtained from adipose tissue studies. The hypercaloric high carbohydrate diet lowered plasma FFA without any changes in plasma glycerol concentration, indicating an increased rate of esterification in adipose tissue in the postabsorptive state. Overfeeding with saturated fat did not alter the plasma FFA concentration, but led to a slight increase in the plasma glycerol concentration which paralleled an increased rate of lipolysis observed in adipose tissue.

It has been clearly shown that overfeeding with glucose induced responses in adipose tissue metabolism different from those caused by overfeeding with fat. Insulin may be a mediator for these different changes in this study. Increased basal plasma insulin was observed during the hypercaloric high carbohydrate feeding, but there was no change in the basal plasma insulin level during overfeeding with fat.
As has already been mentioned, human adipose tissue is responsive to the effect of insulin at physiological dosages, and is modified by the nutritional states of adipose tissue donors. Furthermore, increases in serum insulin concentration in the postabsorptive state were reported in obese subjects overfed with a high carbohydrate diet [Sjöström, 1973b], and in normal weight subjects on a hypercaloric basic diet [Olefsky et al., 1975]. The basal insulin level is also a determining factor for insulin response to an oral glucose load [Bagdade et al., 1967]. On the other hand, a high fat diet fed to rats impaired insulin effects on inhibition of lipolysis [Smith et al., 1974] and on stimulation of glucose utilization by adipose tissue in vitro [Ogundipe and Bray, 1974]. Therefore, the difference in the responses of adipose tissue to overfeeding with glucose or to overfeeding with fat in the present investigation probably reflected different states of insulin secretion and insulin sensitivity in vivo. However, precise reasons for the dietary-induced changes in adipose tissue metabolism in vitro at the absorptive state can not be provided unless carbohydrate tolerance and insulin secretory response are determined.

Liver is a major site for glucose disposal and is central for the regulation of glucose homeostasis in man. Insulin is a primary determinant for glucose metabolism in the liver (see "Role of the Liver in Regulation of Blood Glucose Homeostasis" in Chapter 1, Section 1.6a). Other hormones at physiologic amounts (glucagon, growth hormone and glucocorticoids) probably have permissive actions in controlling carbohydrate tolerance and glucose homeostasis. Dietary factors can modify carbohydrate tolerance, presumably indicating different states of insulin secretion and insulin sensitivity (see Chapter 1, Section 1.6b). Thus, only the insulin secretory response to the standard high
carbohydrate breakfast, during overfeeding regimes in normal subjects, was examined in the present study. The high carbohydrate meal test was used instead of the conventional oral glucose tolerance test, because the adipose tissue studies in vitro were performed 1½ hours following the former meal. The study of carbohydrate tolerance was done during overfeeding in order to examine the states of insulin secretion and sensitivity, which may be responsible for the difference in responses of adipose tissue to carbohydrate and fat content of the hypercaloric diets.

Overfeeding with glucose enhanced carbohydrate tolerance, but had no effect on the insulin secretion recorded after ingestion of the standard meal. This finding indicates that the utilization of carbohydrate by the liver is augmented by increasing sensitivity to the effects of endogenous insulin when normal subjects are overfed with a high carbohydrate diet. This observation is similar to the effects of isocaloric high carbohydrate diets [Anderson et al., 1968; Anderson et al., 1973; Brunzell et al., 1971]. However, in some studies, isocaloric substitution of carbohydrate for dietary fat stimulated early insulin release from the small acute releasable pool of the pancreas [Lerner et al., 1971]. Furthermore, overfeeding with an additional 2,000 calories/day in the form of a mixed diet (P:F:C=15:35:50%) in normal subjects caused a rise in the insulin secretory response to an oral glucose load without any significant change in glucose tolerance [Olefsky et al., 1975]. Increased insulin secretion following an intravenous GTT was also observed in normal subjects overfed with a high carbohydrate diet [Nestel et al., 1970]. These previous findings seem to be divergent from the result of the present study. The reason for this dissimilarity may be in part due to the difference in experimental design, such as the total caloric intake and the carbohydrate
content in the hypercaloric diet. In those studies, subjects were overfed with an additional 1,700-2,000 calories/day, whereas in the present study only an average of 959 calories/day were supplemented to the isocaloric requirement. The other possibility is the metabolic response to overfeeding with glucose in this study (which was observed following ingestion of the high carbohydrate). Carbohydrate was provided in the latter meal mainly as starch, which was shown to have a lesser stimulatory effect on insulin secretion than that induced by glucose or sucrose [Swan et al., 1966]. Although increased insulin levels in the basal state and in response to a glucose load could be induced by overfeeding in normal subjects, there was no evidence to indicate that carbohydrate intolerance or insulin insensitivity did occur [Olefsky et al., 1975]. Adipose tissue is not an important site for carbohydrate disposal. A relatively small amount of a glucose load is utilized by human adipose tissue [Björntorp et al., 1971a]. However, in the present work, overfeeding with a high carbohydrate diet (for 1 or 2 weeks) increased the rate of esterification without altering the rate of lipolysis in adipose tissue, in the absorptive state. These findings were observed both in vivo (indirect approach by measuring plasma FFA and glycerol concentrations) and in vitro. It is possible to conclude that overfeeding with a high carbohydrate diet enhances carbohydrate tolerance and increases sensitivity to endogenous insulin. In addition, the esterification of FFA in adipose tissue is stimulated, presumably reflecting an increased uptake of FFA for storage as triglycerides, by overfeeding with glucose.

Overfeeding with saturated fat had the reverse effect on carbohydrate tolerance and insulin sensitivity, since carbohydrate intolerance was observed without any change in the insulin secretory
response to the standard breakfast during the overfeeding period. There are no data in the literature comparable with the present observations which are similar to the effects of an isocaloric low carbohydrate-high fat diet given to man, previously reported [Hales and Randle, 1963a; Wales et al., 1967; Anderson et al., 1973]. The esterification of FFA in vivo was also stimulated by overfeeding with fat, as judged by the suppression of plasma FFA concentrations in the postabsorptive state and following the standard breakfast. The elevated plasma glycerol level in the absorptive state reflected an impairment of the antilipolytic effect of insulin in adipose tissue in vivo, which was induced by the hyper- caloric high fat feeding. This phenomenon presumably is the cause of the increased rate of lipolysis in adipose tissue in vitro, studied in the absorptive state during feeding with the same dietary regimen in man.

In adipose tissue studies in vitro, overfeeding with fat did not change the rate of esterification in the absorptive state. This was inconsistent with the results of studies in vivo. The possible reason for this aberrant finding may be due to the difference in methodology used for the assessment of the rate of esterification. The rate of re-esterification of FFA in adipose tissue in vitro was indirectly estimated from the difference between the rate of lipolysis and change in FFA in the incubation system. Thus methodological errors in the measurements of glycerol release and FFA concentrations in the tissue and medium could occur, and so prevent the detection of small changes in lipolysis or esterification observed when the end products of adipose tissue metabolism were measured in the plasma. The latter indicated that esterification was enhanced by overfeeding with saturated fat.

It is therefore reasonable to conclude that in man the deposition of an excess amount of fat derived from dietary intake is accompanied by
the following events: a deterioration in carbohydrate tolerance; a
diminution in insulin sensitivity; an augmentation in the rate of
esterification in adipose tissue; and an impairment of the antilipo-
lytic effect of insulin on the lipolysis of adipose tissue triglycerides.
However, the importance of an increased rate of lipolysis on the
deposition and breakdown of fat during overfeeding with fat needs
further investigation. Whether the latter phenomenon is representative
of metabolic events occurring in response to meals throughout the
absorptive state during the day and during the postabsorptive state
remains to be evaluated. Another consideration is whether decreased
insulin sensitivity induced by overfeeding with fat for a longer period
can bring about insulin resistance associated with an increase in body
fat mass.

Obese and non-obese subjects responded to the large caloric
deficits (semi-starvation) for 1-3 weeks with an increase in the break-
down of triglycerides in adipose tissue. *In vitro* the rates of lipo-
lysis, FFA release and net FFA production were greatly increased, but
the rate of esterification was unchanged. The findings *in vitro* were in
accord with the *in vivo* data, since the postabsorptive concentrations of
plasma glycerol and FFA were markedly increased. The present findings
are similar to the report of Kjellberg and Östman [1971], who used the
balance method for the estimation of lipolysis and esterification in
adipose tissue. These observations indicate that only an increased rate
of lipolysis is responsible for the mobilization of fat during a state
of negative caloric balance. This phenomenon was observed both in obese
and non-obese subjects. Thus, obese and non-obese subjects have similar
metabolic responses to the effect of semi-starvation. The only possible
difference in response between the two groups of subjects was a fall in
the elevated tissue FFA concentrations in obese subjects during weight loss. This probably reflected a displacement of excess preformed tissue FFA \textit{in vivo}, whilst there was no change in the previously low concentrations in lean subjects on a semi-starvation diet. Semi-starvation in obese subjects led to a marked fall in the previously elevated concentrations of the basal plasma insulin without significant change in the basal plasma glucose level. When non-obese individuals were placed in caloric deficit, there was a trend towards a decrease in the basal insulin level in the plasma, and a significant fall in the concentration of basal plasma glucose. These data support the concept that the insulin-glucose feedback mechanism is the primary determinant in the regulation of substrate-energy supply during brief starvation, as suggested by Cahill and associates [Cahill \textit{et al.}, 1966; Cahill \textit{et al.}, 1970]. Moreover, it has been demonstrated that the central autonomic nervous system is not important for substrate regulation during early fasting in man [Brodows \textit{et al.}, 1976].

It is well established that metabolic activities in adipose tissue of man or animals are a direct function of adipose cell size (see Chapter 1, Section 1.3). In the present investigation, the rates of lipolysis, esterification were directly proportional to the size of the fat cells. These data were recorded in adipose tissue when subjects were in an isocaloric state following a 14 hour fast. In such conditions, there were also significant positive correlations between most of the parameters of metabolism in adipose tissue. Some of these inter-relationships appear to be modified by the nutritional status, such as in the absorptive state (1½ hours after the standard breakfast) the rate of esterification was not correlated to the fat cell size. The correlation coefficients showed fewer significant inter-relationships
than those calculated in the postabsorptive state. By applying partial correlation analysis, it has been demonstrated that both in the postabsorptive and absorptive states, the rate of lipolysis remains a direct function of fat cell size. In the postabsorptive state, a significant relationship between adipose cell weight and the rate of esterification was demonstrable independently of the rate of lipolysis, the concentration of tissue FFA release and change in tissue FFA concentration. However, in the absorptive state, this relationship was not demonstrated. There was also no direct relationship between the rate of lipolysis and tissue FFA concentration or between the rate of esterification and tissue FFA concentration. It has been purported that in enlarged rat adipocytes, increased lipolysis induces elevation of intracellular concentration of tissue FFA, which in turn stimulates the rate of esterification [DiGirolamo et al., 1974]. Increased rate of lipolysis in enlarged human fat cells was suggested to be due to increases in total triglyceride lipase activity and in the proportion of active lipase to inactive enzyme [Gries et al., 1972]. This suggestion has been supported by a recent study of Arner et al. [1976], who showed that basal levels of cyclic AMP in subcutaneous tissue from obese and non-obese subjects were similar. Tissue levels of FFA in human adipose tissue were also positively correlated with increasing fat cell size [Arner et al., 1976]. However, data of partial correlation coefficient in this study provide no evidence to indicate any relationship between the rate of lipolysis, esterification and tissue FFA in enlarged human fat cells. Lipolysis and esterification are more likely to be separate functions of adipose cell weight. It should be emphasized that correlation or partial correlation analysis does not prove cause and effect, they only serve as a basis for conducting further definite studies.
In conclusion,

(a) isocaloric substitution of carbohydrate for dietary fat has no demonstrable effect on the metabolic activity of adipose tissue \textit{in vitro}, observed after ingestion of a high carbohydrate meal. Some subjects participating in this dietary program were markedly obese. This may partly account for the lack of adipose tissue response to the eucaloric high carbohydrate diet.

(b) fat mobilization (which occurs in a state of negative caloric balance in both obese and non-obese subjects) is regulated solely by an increased rate of lipolysis, which may be mediated by a decrease in the basal insulin level. It is possible that the fall in blood glucose level during the semi-starvation period leads to a diminution in insulin secretion.

(c) overfeeding with carbohydrate stimulates esterification in adipose tissue \textit{in vitro} and \textit{in vivo}, presumably by increasing the sensitivity to endogenous insulin (as shown by the improvement of carbohydrate tolerance without any change in insulin secretion following the standard high carbohydrate meal). Thus a hypercaloric high carbohydrate diet causes increases in the effects of insulin on carbohydrate disposal (which occurs mainly in the liver), and on utilization of glucose for the esterification of FFA in adipose tissue. The stimulation of esterification parallels accelerated uptake of FFA by adipose tissue.

(d) overfeeding with fat decreases the sensitivity to endogenous insulin, as shown by the impairment of carbohydrate tolerance, and thereby causes increases rates of lipolysis in adipose tissue \textit{in vitro} and \textit{in vivo}. There is no effect of the hypercaloric high fat diet on
the rate of esterification observed \textit{in vitro}. However, the \textit{in vivo}
study indicates that the esterification of adipose tissue is enhanced.
The procedure used in studying adipose tissue metabolism \textit{in vitro}, may
not be sensitive enough to detect such changes in the rate of
esterification. This is because the study \textit{in vitro} has inherent
limitations, such as technical errors in measurement of metabolic
variables in small pieces of the adipose tissue samples and multiple
measurements required to obtain the final result.
### APPENDIX 1. CALORIC INTAKE AND DIETARY COMPOSITION (STUDIES OF ADIPOSE TISSUE METABOLISM)

#### a. Effects of High Carbohydrate and High Fat Meals (Studies I and II)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Nutritional State and Type of Diet</th>
<th>On Diet Days</th>
<th>kcal/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Carbohydrate g/day</th>
<th>P:F:C (% of Total Calories)</th>
</tr>
</thead>
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<td>69</td>
<td>724</td>
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<td>145</td>
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<td>2</td>
<td>3,700</td>
<td>90</td>
<td>49</td>
<td>468</td>
<td>126</td>
<td>-</td>
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</table>

<table>
<thead>
<tr>
<th>Subject</th>
<th>Nutritional State</th>
<th>On Diet Days</th>
<th>kcal/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Cholesterol mg/day</th>
<th>Carbohydrate g/day</th>
<th>P:F:C (% of Total Calories)</th>
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<tbody>
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<td>3,700</td>
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<td>160</td>
<td>150</td>
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<td>85</td>
<td>60</td>
<td>7</td>
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</tr>
</tbody>
</table>

* Saturated fat (principal fatty acids 12:0, 14:0 and 16:0).  
† Monosaturated fat (principal fatty acid 18:1).  
‡ Polyunsaturated fat (principal fatty acid 18:2).  
§ Polyunsaturated/saturated fatty acid ratio.
### b. Effects of Isocaloric Substitution of Carbohydrate for Dietary Fat and Overfeeding with Glucose (Studies III and IV)

<table>
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<th>Nutritional State</th>
<th>On Diet Days</th>
<th>kcal/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Carbohydrate g/day</th>
<th>Cholesterol mg/day</th>
<th>P:F:C (% of Total Calories)</th>
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<td>164</td>
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<td>628</td>
<td>164</td>
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<td>146</td>
<td>399</td>
<td>165</td>
<td>103 102 29 536 15:38:47</td>
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<td>141</td>
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<td>4</td>
<td>4,340</td>
<td>116</td>
<td>71</td>
<td>842</td>
<td>201</td>
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<td>34</td>
<td>614</td>
<td>165</td>
<td>191 172 66 144 14: 9:77</td>
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<td>111</td>
<td>34</td>
<td>880</td>
<td>185</td>
<td>250 191 188 66 144 11: 7:82</td>
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### Table: Nutritional Data

#### Subject 11
- **Nutritional State**: Isocaloric standard
- **On Diet Days**: 4
- **kcal/day**: 2,716
- **Protein g/day**: 96
- **Fat g/day**: 111
- **Carbohydrate g/day**: 354, 148
- **Total Starch**: -
- **Glucose**: 109
- **Sucrose**: 82
- **Fructose**: 15
- **Lactose**: 393
- **Cholesterol mg/day**: 14:36:50

#### Subject 12
- **Nutritional State**: Isocaloric high CHO
- **On Diet Days**: 4
- **kcal/day**: 2,707
- **Protein g/day**: 93
- **Fat g/day**: 34
- **Carbohydrate g/day**: 537, 171
- **Total Starch**: 120
- **Glucose**: 136
- **Sucrose**: 96
- **Fructose**: 14
- **Lactose**: 179
- **Cholesterol mg/day**: 13:11:76

#### Subject 13
- **Nutritional State**: Isocaloric standard
- **On Diet Days**: 5
- **kcal/day**: 3,000
- **Protein g/day**: 114
- **Fat g/day**: 135
- **Carbohydrate g/day**: 383, 142
- **Total Starch**: 124
- **Glucose**: 109
- **Sucrose**: 8
- **Fructose**: 459
- **Lactose**: 14:38:48

#### Subject 14
- **Nutritional State**: Isocaloric high CHO
- **On Diet Days**: 6
- **kcal/day**: 2,725
- **Protein g/day**: 105
- **Fat g/day**: 32
- **Carbohydrate g/day**: 524, 171
- **Total Starch**: 182
- **Glucose**: 129
- **Sucrose**: 42
- **Fructose**: 164
- **Lactose**: 15:10:75

#### Subject 15
- **Nutritional State**: Isocaloric high CHO
- **On Diet Days**: 5
- **kcal/day**: 2,775
- **Protein g/day**: 107
- **Fat g/day**: 126
- **Carbohydrate g/day**: 323, 125
- **Total Starch**: 50
- **Glucose**: 109
- **Sucrose**: 39
- **Fructose**: 467
- **Lactose**: 15:40:45

#### Subject 16
- **Nutritional State**: Hypercaloric high CHO
- **On Diet Days**: 9
- **kcal/day**: 3,565
- **Protein g/day**: 116
- **Fat g/day**: 45
- **Carbohydrate g/day**: 706, 147
- **Total Starch**: 160
- **Glucose**: 206
- **Sucrose**: 165
- **Fructose**: 28
- **Lactose**: 252
- **Cholesterol mg/day**: 12:11:77

* Carbohydrates
### c. Effects of Overfeeding with Saturated and Polyunsaturated Fat (Studies V and VI)

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<thead>
<tr>
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<th>On Diet Days</th>
<th>kcal/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Cholesterol mg/day</th>
<th>Carbohydrate g/day</th>
<th>P:F:C</th>
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<td>Total Sat.*</td>
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<td>Poly.‡</td>
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<td>103</td>
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* Saturated fat (principal fatty acids 12:0, 14:0 and 16:0).
† Monounsaturated fat (principal fatty acid 18:1).
‡ Polyunsaturated fat (principal fatty acid 18:2).
∞ Polyunsaturated/saturated fatty acid ratio.
### Effects of Semi-starvation in Obese and Non-obese Subjects (Studies VII and VIII)

<table>
<thead>
<tr>
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<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Carbohydrate g/day</th>
<th>Cholesterol mg/day</th>
<th>P:F:C (% of Total Calories)</th>
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APPENDIX 2. CALORIC INTAKE AND DIETARY COMPOSITION (STUDIES OF CARBOHYDRATE TOLERANCE AND INSULIN SECRETION)

a. Effects of Overfeeding with High Carbohydrate
(Isocaloric High CHO Diet plus an Additional 30% of Calories as Glucose)

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<tr>
<th>Subject</th>
<th>Nutritional State</th>
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<th>kcal/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Carbohydrate g/day</th>
<th>Cholesterol mg/day</th>
<th>P:F:C (% of Total Calories)</th>
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<td></td>
<td></td>
<td></td>
<td>Total Starch</td>
<td>Glucose</td>
<td>Sucrose</td>
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<td>37</td>
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<td></td>
<td></td>
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<td>total</td>
<td>sugar</td>
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<td>Glucose</td>
<td>Sucrose</td>
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<td>Total Starch</td>
<td>Glucose</td>
<td>Sucrose</td>
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<td>Glucose</td>
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<td>128 124 62</td>
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<td>sugar</td>
<td>total</td>
<td>sugar</td>
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b. Effects of Overfeeding with High Fat (Standard Isocaloric Diet plus an additional 30% or 50% of Total Calories as Saturated Fat)

<table>
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<tr>
<th>Subject</th>
<th>Nutritional State</th>
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<th>kcal/day</th>
<th>Protein g/day</th>
<th>Fat g/day</th>
<th>Cholesterol mg/day</th>
<th>Carbohydrate g/day</th>
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<td>Mono.‡</td>
<td>Poly.#</td>
<td>P/S‡</td>
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<td>164</td>
<td>0.06</td>
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</table>

* Saturated fat (principal fatty acids 12:0, 14:0 and 16:0).
† Monounsaturated fat (principal fatty acid 18:1).
‡ Polyunsaturated fat (principal fatty acid 18:2).
§ Polyunsaturated/saturated fatty acid ratio.
APPENDIX 3. TISSUE FFA CONCENTRATIONS AND PARAMETERS OF METABOLISM IN ADIPOSE TISSUE IN THE EUCALORIC STATE*

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<th>Lipolysis</th>
<th>Esterification</th>
<th>( M_{2hr} - M_0 )</th>
<th>( T_0 - T_{2hr} )</th>
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<tbody>
<tr>
<td></td>
<td>( \mu \text{moles/10}^6 ) adipose cells</td>
<td>( \mu \text{moles/10}^6 ) adipose cells/2 hours</td>
<td>( \mu \text{moles/10}^6 ) adipose cells</td>
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</tr>
<tr>
<td></td>
<td>( T_0 ) FFA(^{**} )</td>
<td>Lipolysis</td>
<td>Esterification</td>
<td>( M_{2hr} - M_0 )</td>
<td>( T_0 - T_{2hr} )</td>
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<td>0.274</td>
</tr>
<tr>
<td>13</td>
<td>2.345</td>
<td>1.818</td>
<td>1.690</td>
<td>0.363</td>
<td>0.235</td>
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<tr>
<td>14</td>
<td>1.855</td>
<td>1.231</td>
<td>1.678</td>
<td>-0.072</td>
<td>0.375</td>
</tr>
<tr>
<td>15</td>
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<td>1.629</td>
<td>2.655</td>
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</tr>
<tr>
<td>16</td>
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<td>3.697</td>
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</tr>
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<td>17</td>
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<td>1.643</td>
<td>1.474</td>
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<tr>
<td>18</td>
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</tr>
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<td>19a</td>
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<td>1.251</td>
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</tr>
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<td>b</td>
<td>9.998</td>
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<td>1.610</td>
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<td>0.829</td>
</tr>
<tr>
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<td>6.873</td>
<td>1.063</td>
<td>1.412</td>
<td>0.316</td>
<td>0.665</td>
</tr>
</tbody>
</table>
* Values are given as means of 2 or 3 adipose tissue biopsies.
† Tissue FFA concentration after 30 minutes of preincubation.
# Parameters of adipose tissue metabolism recorded at the end of 2 hour incubation.
/ These data were used for the analysis of correlation coefficients of relationship between the adipose cell size and metabolic parameters of adipose tissue in the postabsorptive and absorptive states respectively.


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