THE EFFECT OF DRUGS UPON METABOLIC CHANGES RESULTING FROM VARIOUS STRESSES WITH PARTICULAR REFERENCE TO EXERCISE

by

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W. R. Sulaiman

CONTENTS

ABBREVIATIONS

The following abbreviations are used throughout the text of this thesis:

ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
ACTH	Adrenocorticotrophic hormone
cal	Calorie
CP	Creatine phosphate
FFA	Free fatty acids
GK	Glycerokinase
ht	Height
hr	Hour
HBDH	Hydroxybutyrate dehydrogenase
HGH	Human growth hormone
IRI	Immuno-reactive insulin
iv	Intravenous
LDH	Lactate dehydrogenase
Log	Logarithm
NAD (NADH)	Nicotinamide adenine dinucleotide (reduced)
min	Minute
PCA	Perchloric acid
PEP	Phosphoenol pyruvate
PK	Pyruvate kinase
R	Respiratory exchange ratio
rpm	Revolution per minute
SEM	Standard error of the mean
wt	Weight

Units used

l	Litre	
ml	Millilitre	$- 10^{-3}$L
µl	Microlitre	$- 10^{-6}$L
kg	Kilogram	
g	Gram	
mg	Milligram	$- 10^{-3}$g
µg	Microgram	$- 10^{-6}$g
ng	Nanogram	$- 10^{-9}$g
pg	Picogram	$- 10^{-12}$g
kp	Kilopond	
m	Meter	
cm	Centimeter	$- 10^{-2}$m
nm	Nanometer	$- 10^{-9}$m
M	Mol or Molar	
µ mol	Micromol	$- 10^{-6}$mol
µ equiv	Equivalent	$- 10^{-6}$equiv
U	Unit	
mU	Milliunit	$- 10^{-3}$U
µU	Microunit	$- 10^{-6}$U
C	Curie	
µC	Microcurie	$- 10^{-6}$C

PREFACE

In October, 1970, I enrolled as a research student in the Department of Biochemistry, University of Glasgow, with Professor R.M.S. Smellie as my professorial supervisor. On his advice I joined Dr. R.H. Johnson's research group to work under his supervision in the University Department of Neurology.

Dr. Johnson suggested, in view of my previous experience as a pharmacist, that I should begin by studying the metabolic changes with fenfluramine, a drug prescribed for the treatment of obesity. This study was carried out with a comparative study on the metabolic changes during therapeutic starvation for obesity. On acceptance in 1972 as a Ph.D. student I extended my research programme to examine the metabolic effects of exercise and their alteration by drugs, including fenfluramine, alcohol and nicotinic acid.

It was therefore necessary to investigate biochemical and hormonal changes during exercise in normal man. During exercise, mobilization of fuels varies with physical fitness, nutritional state and pathological condition. Differences may be secondary to changes in hormonal production during exercise. The effects of athletic fitness, obesity and dietary restriction were studied in order to gain further knowledge of variations which may occur in normal man. In addition patients with metabolic and hormonal abnormalities were studied for comparison. A case of ocular-myopathy was also studied.

These studies have necessitated the development of techniques to measure human growth hormone and immunoreactive insulin. I have therefore gained expertise in biochemical, enzymatic and radio-immunological techniques. All assays of blood metabolites and hormones have been performed by myself and the results form the material for this thesis.

INTRODUCTION

1. Preface Knowledge of the regulation of metabolic pathways has developed at a remarkable rate during the past decade and has made it possible to study the storage, synthesis and utilization of various fuels concerned in energy production. Experimental studies on physical activity in man and in animals have provided much information on fuel mobilization and utilization. Such basic information makes it possible to study metabolic control in clinical disorders and the effect of various drugs. Most of the research in metabolic and hormonal regulation has been carried out relatively recently and some theories are still controversial. I have, therefore, summarized the available experimental evidence in this introduction and explained the reasons for the development of the studies reported in this thesis.

2. Historical background.

The human body continually converts chemical energy obtained from food into work and heat. An inherent difficulty in studying normal metabolism arises because it is not possible to separate accurately the processes of catabolism from those of storage and synthesis. Thus skeletal muscle not only performs mechanical work but also contains important reserves of carbohydrate and some fat. The energy used by muscle in performance of work is derived from chemical reactions going on within the cells. The nature of these reactions was the subject of much experimentation and controversy during the latter half of the nineteenth century, but quantitative and reproducible results were not obtained until the classical work of Fletcher and Hopkins (1907). They showed clearly that fatigue is accompanied by lactic acid production. Later, in 1914, Parnas and Wagner showed that lactic acid is derived from

glycogen. For forty years physiologists concentrated upon the reaction in which lactic acid was formed from carbohydrate and considered it the only energy-providing reaction. This remainod unchallenged until the discovery in 1927 by the Eggletons, of "phosphagen". Later identified as CP and ATP by Lohmann in 1930. Their discoveries made it possible for Lundsgaard and other workers to demonstrate that muscle contraction is proportional to the breakdown of phosphagens. In addition to extensive studies of the energy supplies for contraction, work has also been progressing in the role of stores of fuel in muscle in energy metabolism.

It was long thought that the primary if not exclusive fuel for muscle metabolism was carbohydrate. In 1938, Lundsgaard summarized the available information responsible for this hypothesis and in addition cited the work of Blixenkrone-Möller, 1938) which had demonstrated that ketone-bodies were utilized by the tissue of a perfused hind-leg of the cat. It therefore appeared that other substrates might be involved in muscle metabolism. Christensen and Hansen provided evidence in 1939 to suggest that fat could also be metabolised by muscle. Their evidence was derived primarily from low R values in exercising subjects and in isolated muscle preparations. Ten years later Geyer and co-workers (1949) provided more direct evidence to indicate that fatty acids can be utilized by isolated skeletal muscle. These discoveries threw new light on many earlier observations and increased interest came to be shown in the study of the relation of fat and carbohydrate metabolism in clinical situations.

3. Carbohydrate metabolism - general considerations.

In carbohydrate metabolism the rate of glucose entry into the

cell and its subsequent phosphorylation with ATP to glucose-6-phosphate is the preliminary step for its utilization. Glucose-6-phosphate formed may either be used for glycogen synthesis or catabolised to pyruvate through the glycolytic pathway. The sequence of events up to the formation of pyruvate are identical in the presence or absence of oxygen. Thereafter the products vary with the available oxygen supply and pyruvate is either reversibly converted to lactate or decarboxylated to form acetyl CoA. The oxidation of glucose to pyruvate in the Embden-Meyerhof pathway is achieved by hydrogenation with the use of NAD-NADH which act as electron transfer agents.

The liver is the principal source of glucose entering the circulation. Glucose output from the liver is regulated partly by a homeostatic action of the liver itself and partly by hormonal influences. When the level of blood glucose falls, the formation of glucose from glycogen (glycogenolysis) or from 2 and 3 carbon fragments (gluconeogenesis) is increased. A reverse process occurs when blood glucose tends to rise. Various hormones influence hepatic glucose output directly, in consequence of their effect on hepatic metabolism and also as a result of their metabolic actions in other tissues. Insulin is known to cause a fall in blood glucose. This results from increased peripheral glucose utilization with increased glycogen deposition in liver and muscle, and increased fat synthesis combined with lowered rates of gluconeogenesis and hepatic glycogenolysis. The hormones adrenaline, nor-adrenaline, HGH, glucagon and ACTH are known to stimulate the hydrolysis of both fat and glycogen. The primary effect of each may be to increase the production of cyclic AMP by activating the adenyl cyclase system (Sutherland et al., 1968).

All have a diabetogenic effect, either directly by inhibiting the release of insulin from the pancreas (e.g. adrenaline and nor-adrenaline; Williams and Ensinck, 1966), or indirectly by decreasing the peripheral uptake of glucose and its subsequent oxidation, perhaps by enhancing fat mobilization (e.g. HGH, Randle et al., 1963). It appears, therefore, that these hormones provide reserve capacity to regulate fat and carbohydrate metabolism.

4. Fat metabolism - general considerations

In the breakdown process, fat in the form of triglyceride stored in adipose tissues is hydrolysed by lipase to FFA and glycerol. Because the enzyme glycerokinase is low in activity in adipose tissue, glycerol diffuses out into the plasma from which it is withdrawn and metabolized by the liver and kidney to α-glycerophosphate. The FFA formed by lipolysis can be either re-esterified in the tissue and stored as triglyceride or oxidized by the liver and peripheral tissue through β-oxidation to acetyl CoA. The preliminary step for FFA oxidation requires the availability of ATP and CoA ester to form long chain acyl CoA, which then undergoes a series of reactions catalysed by oxidative mitochondrial enzymes and co-factors to acetyl CoA. The fate of acetyl CoA may depend on the availability of carbohydrate. Glucose enhances the complete oxidation of acetyl CoA to CO_2, water and ATP, but when the supply of glucose is short as in starvation, ketone-body formation from acetyl CoA is increased.

The rate of release of FFA from adipose tissue is affected by many hormones that influence either the rate of esterification or the rate of lipolysis. Insulin by increasing the oxidation of glucose,

inhibits the release of FFA and enhances the synthesis of triglycerides. All these effects are dependent on the presence of glucose in the medium and can be explained, therefore, on the basis of increased uptake of glucose into adipose tissue. Insulin in adipose tissue also inhibits the activity of the enzyme lipase, reducing not only the release of FFA but also of glycerol. Other hormones such as adrenaline, nor-adrenaline, glucagon, HGH and ACTH act to stimulate the rate of lipolysis by activating the adenyl cyclase system (Sutherland et al., 1968).

5. Supply and use of fat and carbohydrate.

Fat and carbohydrate are the major fuels and with a few exceptions notably brain, the various tissues of the body can burn either efficiently, but the amounts used tend to vary under different nutritional pathological and with changing demand for energy during exercise.

5a. Nutritional state

1. Post prandial: Carbohydrate as glucose after absorption is either stored as glycogen in liver and muscle or enters the systemic circulation. The energy rich FFA, like glucose enters the tissues rapidly and serves as a readily available energy source. During food absorption and as a result of increased insulin secretion and uptake of diet-derived glucose into several tissues, the uptake and storage of fat in adipose tissue is promoted while mobilization is inhibited.

2. Post-absorptive: Owen and Reichard (1971) have found that fasting of short duration, depletes stored glycogen whereas stores of triglycerides in adipose tissue can continue to supply energy needs for one to two months.

In starvation as the rate of fat mobilization increases, the rate of ketone-body production also increases. In this situation, the rise of blood ketone-bodies may alter the relative contribution of metabolic fuels for working muscle, since ketone-bodies have been shown to inhibit glucose oxidation in muscle and impairs the responsiveness of this tissue to insulin (Garland and Randle, 1964).

5b. Exercise: During muscular exercise the immediate fuels include high energy phosphate, mainly CP and ATP and the conversion of glycogen to lactate (Hultman, 1967). Use of stored glycogen, evidenced by release of lactate, occurs at the onset of exercise (Pernow and Wahren, 1962). This appears to diminish after a few minutes, presumably as blood flow increases (Jorfeldt, 1970). The use of glycogen seems to depend upon the extent to which other fuels, particularly FFA from blood can supply energy needs. Their entry into the blood from adipose tissue increases rapidly with exercise (Havel et al., 1963; Havel et al., 1964). Adrenergic stimulation of adenyl cyclase system in adipose tissue, appears to be mainly responsible for release of FFA (Havel, 1965). Levels of HGH in plasma also rise with exercise (Hunter et al., 1964). Increased secretion of HGH may contribute to fat mobilization after exercise of long duration, but is unlikely to be responsible for rapid mobilization (Hartog et al., 1967). Increased sympathetic activity could inhibit secretion of insulin (Porte and Williams, 1966) and increase the production of glucose, lactate and pyruvate from liver and muscle (Young et al., 1967).

Subjects in athletic training however tend to have a smaller rise of blood FFA and lactate concentrations during exercise and lower

blood ketone-bodies in the post-exercise period (Johnson et al., 1969). Training has been found to induce changes in pathways regulating fat and carbohydrate oxidation (Mole et al., 1971; Oscai and Holloszy, 1971). Since most of the metabolic processes which are known to be altered by physical training are influenced by endocrine activity, it is possible that training modifies the release of hormones including HGH and insulin. Although an increase during exercise in plasma concentration of HGH and a decrease in IRI concentration have been reported (Roth et al., 1963; Hunter and Greenwood, 1964; Hunter and Sukkar, 1968), there is however little information about the changes of these hormones when related to physical fitness.

6. Pathological states:

6a. Obesity. Studies in man have provided evidence that individuals who exercise regularly have a greater lean body mass than sedentary individuals of the same weight (Parizkova, 1963). In studies on growing rats, animals which were exercised regularly were found to be leaner than sedentary animals living on a restricted diet (Crews et al., 1969). These observations suggest that lack of physical training leads to a mild relative degree of obesity which would alter the relative contribution of fat and carbohydrate as fuel. In obesity, fat synthesis is increased, and the rate of fat mobilization and oxidation appears to be decreased (Gordon et al., 1962). Obese individuals appear to handle a glucose load quantitatively differently from normal subjects. Glucose tolerance is often lower in obese subjects and this may reflect differences in peripheral uptake of glucose which is often defective in obesity (Butterfield et al., 1965). This abnormality, with the raised plasma

insulin levels frequently seen in obese subjects (Karam et al., 1963) would facilitate lipogenesis in adipose tissue. Higher concentrations of plasma FFA than normal are found in obese subjects after overnight fasting (Opie and Walfish, 1963) although HGH levels are low (Beck et al., 1964). It would seem that energy in obesity results much more from fat oxidation, although fat stores in obese subjects are found to be less sensitive to the effect of starvation (Beck et al., 1964). During starvation in the obese the increase of plasma FFA is frequently less which may be due to decreased adrenergic activity (Januszewicz et al., 1967) or to lower HGH concentrations (Beck et al., 1964). Obese subjects also produce ketone-bodies at a slower rate during starvation and develop less ketosis (Kekwick et al., 1959).

6b. Diabetes mellitus: Diabetes mellitus is due to insufficiency of insulin relative to the requirements of the tissues. The juvenile diabetic has little detectable circulating insulin and his pancreas fails to respond to a glucose load. On the other hand the maturity onset diabetic may show insulin resistance, and with a glucose load has continued elevation of glucose concentrations. He may however secrete more insulin for a given glucose load than do normal subjects (Seltzer et al., 1967). Thus, plasma glucose/insulin ratios are usually much higher than normal in these subjects (Hales et al., 1968).

Insulin deficiency results in an abnormal balance of hormonal control. Thus the relatively insulin deficient subject is in a state of hormonal imbalance favouring the action of corticosteroid, HGH and glucagon which add to the stimulation of gluconeogenesis, lipolysis and decreased intracellular metabolism of glucose (Randle et al., 1963).

6c. Muscle diseases: A number of chronic myopathic disorders characterised by apparently specific histo-pathological abnormalities in voluntary muscle have been described. These have included myophosphorylase deficiency (McArdle's syndrome) and other conditions in which there seems to be structural and functional abnormalities in the muscle mitochondria (McArdle, 1951; Luft et al., 1962; Shafiq et al., 1968; Bradley et al., 1969). McArdle (1951) has found that individuals with myophosphorylase deficiency cannot utilize their store of glycogen in skeletal muscle effectively. Lactate therefore does not accumulate during exercise or hypoxia.

In one patient with an abnormality of the mitochondria (Bradley et al., 1969) there were excessive amounts of FFA in the muscle fibres and a defect in FFA oxidation was suggested. It, therefore, seems likely that alteration in the supply of fuel for energy under these conditions may cause a considerable effect on substrate utilization as well as the ability to perform physical activity.

7. Pharmacological agents affecting fat and carbohydrate metabolism:

In recent years many studies have concentrated on the use of drugs as a tool for the elucidation of the biochemical mechanisms involved in regulation of fat and carbohydrate metabolism. Two factors have contributed towards this progress. One is the improvement of analytical methods, the other is the development of pharmacological agents which directly affect biosynthesis, degradation, storage or release of metabolic substrates. In general, liver and peripheral tissues are primarily responsible for the supply and utilization of fat and carbohydrate. Some drugs act by interfering with one or more metabolic

pathways. Since most of the metabolic processes in liver and peripheral tissues are influenced by hormones, some drugs may also act by altering endocrine activity.

7a. Fenfluramine: This drug is a derivative of amphetamine which has had an extensive clinical use for the management of obesity. It was originally suggested that its action may be dependent on appetite suppression, but evidence has accumulated that the drug also has an action on tissue metabolism. It appears to improve the peripheral uptake of glucose (Butterfield and Whichelow, 1968), although the basis for this action is not clear. It also causes a fall in concentrations of serum triglyceride (Pawan, 1969). During therapy with fenfluramine concentrations of plasma FFA, glycerol and total ketone-bodies increase (Pawan, 1969). Whether these effects of fenfluramine are direct or secondary to dietary restriction or to possible effect on centres regulating hormonal activity is not certain. Observations in animals have indicated that the hypothalamus is a possible site for the action of the drug (Anand, 1971).

7b. Nicotinic acid: This drug is a potent agent in reducing fat mobilization from adipose tissue (Carlson and Oro", 1962) and this may in turn affect the utilization of glucose by peripheral tissues. Although nicotinic acid inhibits the activity of adipolytic lipase both *in vivo* and *in vitro*, the precise details of its action on fat metabolism are not clear. The effect of nicotinic acid on carbohydrate metabolism is even less defined and both hypo- and hyperglycemia have been reported (Mirsky *et al.*, 1957; Parsons, 1961).

7c. Ethyl alcohol: Ethanol exerts several different types of action on body metabolism. Alcohol is mainly metabolized in the liver by enzymatic mechanisms which are common to the metabolic pathways of fat and carbohydrate metabolism, particularly those dependent on the availability of NAD and NADH. Following either acute or chronic administration of ethanol to mammals, the hepatic concentrations of NAD and NADH change, shifting the redox state of the liver to a more reduced state (Gordon, 1972). This may, in turn, reduce the production of glucose and increase the synthesis of fat (Hawkins and Kalant, 1972). Other interesting metabolic effects of alcohol include its ability to depress secretion of posterior pituitary antidiuretic hormone and stimulation of corticosteroid secretion (Jenkins and Connolly, 1968; Bellet et al., 1970). Since alcohol is believed by some investigators to activate hypothalamic, pituitary and adrenal activity, it is possible that alcohol also interferes with the release of HGH, as the hypothalamus regulates the release of HGH (McCann, 1970; Martin, 1973).

8. Scope of the present studies.

Tolerance tests:

8a. Acetoacetate tolerance test: Following the administration of ketones to animals or man, a decrease in the concentration of blood glucose and FFA has been observed by many workers (Madison et al., 1964; Björntorp and Schersten, 1967; Balasse and Ooms, 1968; Senior and Loridan, 1968). This effect of ketones has been accepted as compatible with stimulation of the release of insulin (Madison et al., 1964). Although a rise in plasma insulin after administration of ketones has

been demonstrated in dogs, the relationship between ketone-bodies and insulin release is less clearly defined in man. The effect of aceto-acetate administration upon fat and carbohydrate metabolism in man was therefore studied to determine its effect on insulin and growth hormone release at rest in normal subjects.

8b. Glucose tolerance test: This was performed before and after the administration of fenfluramine on patients with obesity and of nicotinic acid in normal subjects in order to investigate the action of these drugs on carbohydrate metabolism in relation to insulin release.

8c. Observations after an overnight fast and during dietary restriction: Serial measurements of FFA, glucose, glycerol, aceto-acetate and 3-hydroxybutyrate were made in two groups of obese subjects. One was taking fenfluramine, the other were maintained on reduced diet. This was carried out in order to examine whether fenfluramine action on fat and carbohydrate metabolism is related to a reduction in dietary intake.

8d. Exercise studies: In the past decade while considerable interest and effort have been devoted to increasing our understanding of factors regulating energy metabolism during exercise in normal subjects, little information is available, however, on the changes during exercise in patients with altered metabolic and nutritional situations such as obesity and starvation. Exercise studies were designed to compare the patterns of blood metabolites and hormonal responses to exercise between physically well trained subjects and healthy untrained subjects. Comparative studies between patients with obesity and normal non-obese subjects were also carried out. The reason for carrying out these investigations was both to establish the usual response of blood

metabolites and hormones to exercise and to measure these responses after the administration of drugs such as fenfluramine, nicotinic acid and alcohol.

Exercise was chosen because it produces considerable demands on fat and carbohydrate metabolism and changes in endocrine activity. In this situation any metabolic or hormonal effect of pharmacological agents, pathological and altered nutritional states could be expected to be magnified.

METHODS -- A SUMMARY

A full account of all methods used in the present work appears in Appendices I and II. This section gives a short description of the main techniques.

(a) Experimental Procedures

1. Catheterization: The subjects undergoing clinical investigations were brought to the laboratory between 09.00-10.00 hours after an overnight fast. A catheter (gauge 18-20) was placed in the antecubital vein under local anaesthesia, flushed with saline (0.9%) and strapped to the forearm.

2. Exercise: After catheterization, two resting blood samples were taken. The subjects then exercised on either a bicycle ergometer (Elma Schönander Constant load ergometer EM 369) or a treadmill for a period of 20-30 min, followed by a period of rest of 90 min. Heart rate was recorded during the investigation and for a further 15 min using miniature chart electrodes with an electrocardiograph. Blood samples were taken at 5 min intervals during exercise and then at 15, 30, 60 and 90 min afterwards.

3. Glucose Tolerance Test: 200 ml of 25% w/v glucose was taken orally in about 2 min. The subjects remained at rest throughout the test. Venous blood samples were taken beforehand and at 30 min intervals after glucose ingestion for 180 min.

4. Acetoacetate Tolerance Test: 200 ml of 0.4M sodium acetoacetate, prepared according to the method of Krebs and Eggleston (1945) was taken orally. The subjects remained at rest throughout the test. Venous blood samples were taken beforehand, after 40 min and then at subsequent 20 min intervals for a further hour.

(b) Biochemical techniques

Venous blood (14 ml) was taken on each occasion and divided into two parts, 4 ml of this blood was immediately deproteinized with PCA. The remaining 10 ml of blood was heparinized and the plasma was separated later from the cells by centrifugation. The acid extract of the first sample was neutralized with KOH and used for the estimation of metabolites in the blood. Lactate, pyruvate, acetoacetate, 3-hydroxybutyrate and glycerol were analysed by enzymatic methods. The disappearance of NADH was measured at 340 nm using a spectrophotometer. Glucose was estimated by a colorimetric method using glucose oxidase and ortho-dianisidine as an indicator. The plasma sample was analysed for FFA by a colorimetric method after chloroform extraction and soap formation with a copper salt. Details of the methods are given in Appendix I.

(c) Hormonal assays

A radio-immuno-assay has been developed for the measurement of both HGH and IRI in plasma. In the HGH estimation a double antibody technique was modified in order to precipitate the bound HGH. Albumin coated with charcoal was used to absorb free insulin. The immunological activities of both hormones were derived from graphs of results from control samples in which the percentage of hormone which was bound had been plotted against the log of the total concentration of the hormone. Details of the hormonal assays are given in Appendix II.

CHAPTER 1

TRAINED CYCLISTS COMPARED WITH NORMAL SUBJECTS

INTRODUCTION

Several drugs which affect fat and carbohydrate metabolism have been used in the management of either obesity or diabetes, disorders in which such metabolism is altered. Among the drugs are derivatives of amphetamines and biguanides. Their modes of action are still not fully understood. There is also little information about their effect on metabolism during exercise. In order to elucidate these actions in patients suffering from metabolic abnormalities, such as obesity and diabetes, it is necessary to be aware of the metabolic changes which occur during exercise in normal subjects. The study of exercise is of value because metabolic processes and the mechanism regulating the fuel supply are accelerated.

Normal subjects are not, however, homogeneous for their degree of physical fitness varies considerably. In order to investigate the range in normal subjects, I have studied well-trained athletes as well as untrained subjects. In previous studies athletes have been shown to have lower concentrations of blood lactate and pyruvate during exercise (Robinson and Harmon, 1941; Holmgren and Ström, 1959; Cobb and Johnson, 1963; Saltin and Karlsson, 1971), and lower concentrations of plasma FFA and blood ketone-bodies in the post-exercise period (Johnson et al., 1969; Johnson and Walton, 1972). Nevertheless they had higher blood glucose and glycerol concentrations during exercise than untrained subjects (Rennie et al., 1974). As there are differences in the rate of fuel mobilisation, endocrine activity is also probably altered as a result of athletic training. Although some alterations, in the response of

catecholamine (von Euler and Hellner, 1952) and HGH (Sutton et al., 1969) as a result of physical training, are known during exercise, there is, however, little information about changes in the plasma IRI in relation to physical fitness.

To establish the hormonal changes with exercise further, I have studied trained and untrained subjects during exercise of an increasing severity. This investigation was carried out in conjunction with M.J. Rennie who has reported the catecholamine changes in his thesis, but I was alone responsible for the observations which follow.

METHODS

Six male racing cyclists and six male untrained subjects (table 1.1) have been studied. They were investigated on two occasions. On the first occasion, the maximum work capacity for all the subjects was determined after cycling at work loads increasing by 100 kpm every minute until exhaustion. On the second occasion, the subjects were studied during and after 32 minutes of exercise on a bicycle ergometer. They were studied between 1700 and 1900 hr and had not eaten since mid-day. The exercise consisted of continuous cycling at 60 rpm for four periods of 8 minutes, each at work loads were approximately 30, 45, 60 and 75 per cent of maximal capacity. Blood samples were taken from a catheter placed in an antecubital vein. One resting sample was taken and further samples were also taken at 8 min intervals during exercise and then at 5 min after the exercise. Samples were treated as described in appendices I and II and analysed for lactate, pyruvate, glucose, glycerol, ketone-bodies, FFA, IRI and HGH. Heart rate was recorded with a continuous electrocardiogram.

Table 1-1: Individual data on age, height, weight, maximum work load and heart rate of 6 trained cyclists and 6 untrained subjects

(a) Trained cyclists

	Age yr	Height cm	Weight Kg	% increase above ideal weight	Maximum work load kpm	Heart rate beats/min	
						Rest	End of exercise
M.D.	27	182	79	8	2500	58	193
D.M.	27	168	62	3	2100	48	195
A.T.	27	185	80	6	2300	96	192
W.M.	26	168	60	2	1900	70	196
E.McD.	22	172	67	3	2100	55	198
A.McD.	26	175	63	2	1900	48	198
Mean	26	175	68	4	2133	63	195
SEM	± 1	± 3	± 4	± 1	± 95	± 7	± 1
(b) Untrained subjects							
W.G.	25	182	65	3	1400	84	196
M.R.	27	188	90	17	1600	88	182
S.H.	28	186	68	4	1700	81	185
S.S.	28	176	78	16	1500	94	192
W.S.	33	168	75	18	1200	85	182
D.P.	33	178	73	8	1400	67	185
Mean	29	180	75	11	1466	83	186
SEM	± 1	± 4	± 4	± 3	± 71	± 4	± 2

RESULTS

The trained subjects were relatively younger and somewhat shorter and lighter than the untrained group (table 1:1). Significantly greater work loads were achieved by the trained subjects with relatively similar maximum heart rates to that of the untrained subjects.

FFA and glycerol (table 1.2); Resting FFA concentrations were similar in both groups. Exercise caused a slight fall in FFA at 8 min in the control group, but the fall was more marked for the cyclists whom it was observed for 16 min of exercise. In both groups FFA concentrations rose after the initial fall both in the remaining part of exercise and at the end of the investigation, but the rise was greater for the controls (66% of resting) than the cyclists (8% of resting).

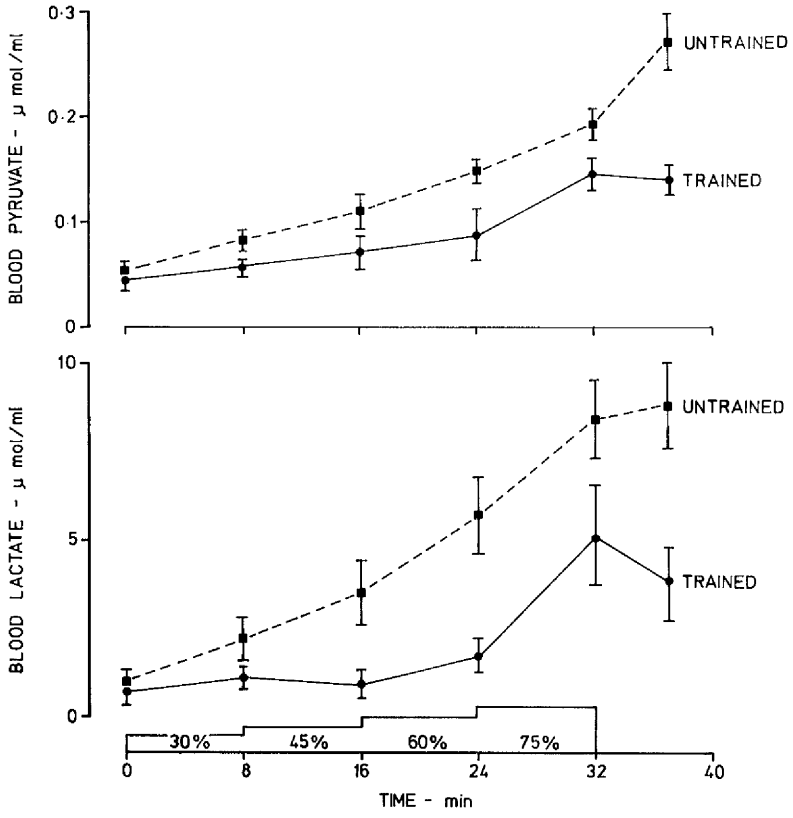
Glycerol concentrations were similar at rest in both groups, but with exercise there was a rapid elevation in the cyclists. In both groups the highest concentration was achieved at the end of the investigation, but the values were higher in the cyclists 0.308 μmol/ml (± 0.03 μmol SEM) compared with 0.158 μmol/ml (± 0.02 μmol SEM) in the controls.

Lactate and pyruvate (fig 1.1); Resting concentrations of blood lactate were similar in both the trained and untrained subjects. After 16 minutes of exercise, the mean lactate values were 0.98 μmol/ml (± 0.23 μmol SEM) and 3.65 μmol/ml (± 0.83 μmol SEM) respectively. At the end of the exercise period, the cyclists had a mean value of 5.04 μmol/ml (± 1.13 μmol SEM) and in the untrained subjects, lactate concentrations had risen to 8.41 μmol/ml (±0.97 μmol SEM). After exercise lactate concentrations fell

			E X E R C I S E				
Time (min)		0	8	16	24	32	37
plasma FFA (μequiv/ml)	U	1.02 ± 0.08	1.00 ± 0.12	1.04 ± 0.01	1.12 ± 0.09	1.30 ± 0.09	1.70 ± 0.16
	T	0.98 ± 0.04	0.90 ± 0.06	0.96 ± 0.02	1.02 ± 0.04	1.02 ± 0.05	1.06 ± 0.07
		NS	P<0.05	P<0.05	NS	P<0.01	P<0.001
Blood glycerol (μmol/ml)	U	0.096 ± 0.010	0.098 ± 0.010	0.099 ± 0.010	0.112 ± 0.013	0.119 ± 0.013	0.158 ± 0.020
	T	0.083 ± 0.003	0.120 ± 0.012	0.166 ± 0.027	0.213 ± 0.028	0.273 ± 0.037	0.308 ± 0.030
		NS	P<0.05	P<0.01	P<0.001	P<0.001	P<0.001

Table 1-2: Plasma FFA (μequiv/ml) and blood glycerol (μmol/ml, mean ± SEM) during and after exercise in 6 trained cyclists (T) and 6 untrained subjects (U). All subjects exercised for four successive periods of 8 min, each at 30%, 45%, 60% and 75% of their maximum capacity.

Fig. 1-1. Blood lactate and pyruvate (μmol/ml, mean ± SEM) during and after exercise in 6 trained cyclists (●——●) and 6 untrained subjects (■——■). All subjects exercised for four successive periods of 8 min, each at 30%, 45%, 60% and 75% of their maximum capacity.


BLOOD PYRUVATE - μ mol/ml
0·3
0·2
0·1
0
UNTRAINED
TRAINED
BLOOD LACTATE - μ mol/ml
10
5
0
UNTRAINED
TRAINED
30%
45%
60%
75%
0
8
16
24
32
40
TIME - min

in the cyclists, but continued to rise in the untrained subjects so that the difference during the first few minutes after ceasing exercise was considerable.

The changes in pyruvate concentrations were similar to those of lactate both during and after exercise.

Glucose (fig 1.2) At rest, blood glucose concentrations were not significantly different between the trained and untrained groups ($p > 0.05$). After 24 minutes of exercise, the mean glucose values were 116 mg% ($\pm$ 7 mg SEM) and 85 mg% ($\pm$ 7 mg SEM) respectively. At the end of the exercise period, the concentrations were greatly elevated in the cyclists, whereas there was a much smaller increase in the value for the untrained subjects ($p < 0.01$). The differences between the two groups were maintained in the post-exercise period.

Plasma IRI (fig 1.2) Resting concentrations of plasma IRI were similar in both groups. The concentrations fell during exercise in the untrained subjects, but remained unchanged in the cyclists so that the differences in the concentrations of IRI between the two groups were significant throughout the exercise ($p < 0.01$). After exercise the concentrations rose considerably in both groups, but the rise was much greater in the untrained subjects.

Plasma HGH (fig 1.3) At rest plasma HGH concentrations were not significantly different between the two groups ($p > 0.05$). Exercise caused a rise in the concentrations of HGH in both groups, but the increase was much greater in the untrained subjects. At the end of the investigation the concentrations fell in the cyclists whereas in the

Fig. 1-2. Blood glucose (mg/100 ml) and plasma IRI (μ units/ml, mean ± SEM) during and after exercise in 6 trained cyclists (○ ——— ○) and 6 untrained subjects (■ ——— ■). All subjects exercised for four successive periods of 8 min, each at 30%, 45%, 60% and 75% of their maximum capacity.

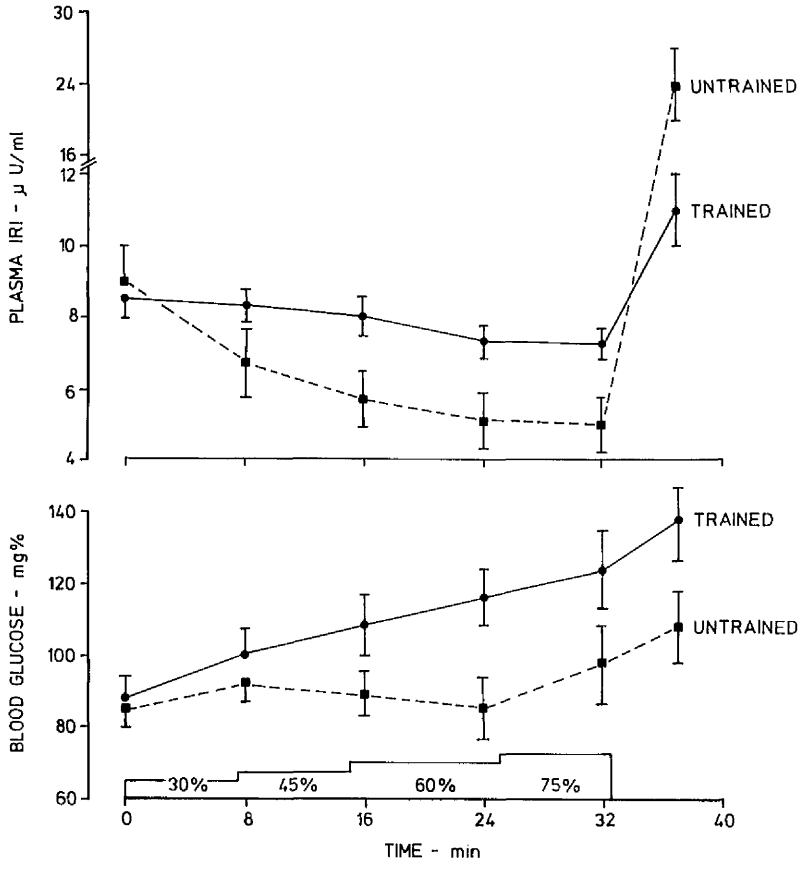
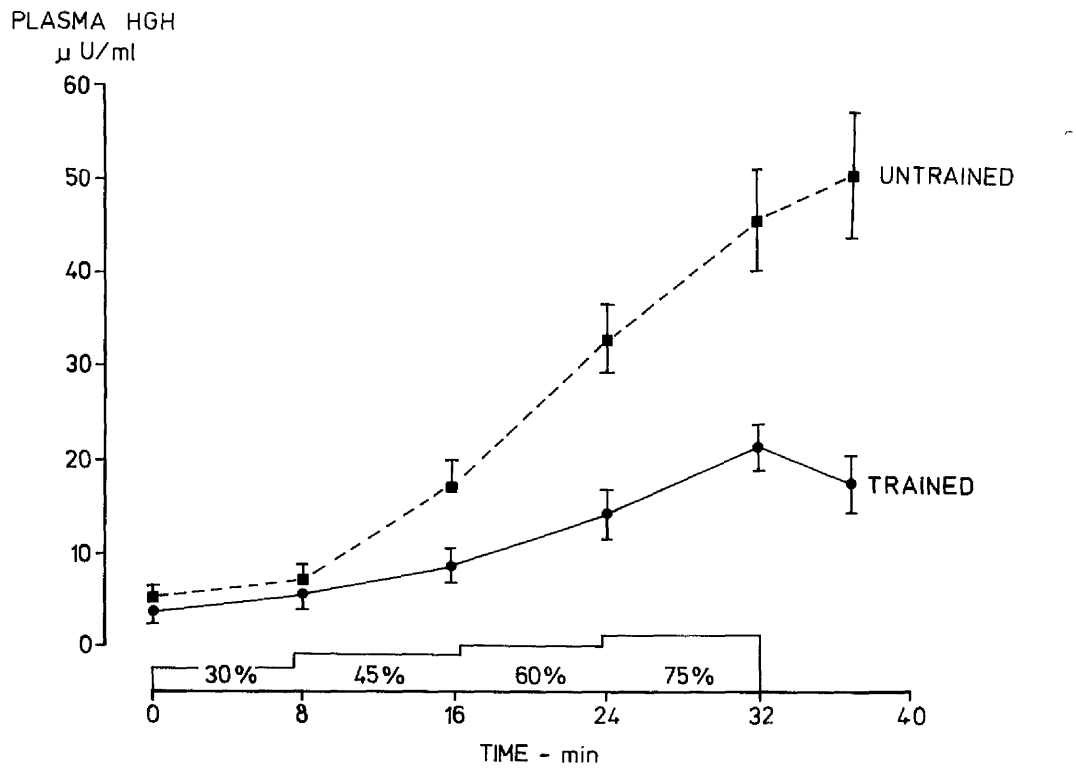

PLASMA IRI - μ U/ml
30
24
16
12
10
8
6
4
UNTRAINED
TRAINED
BLOOD GLUCOSE - mg%
140
120
100
80
60
TRAINED
UNTRAINED
30%
45%
60%
75%
0
8
16
24
32
40
TIME - min

Fig. 1-3. Plasma HGH (μ units/ml, mean ± SEM) during and after exercise in 6 trained cyclists (● ——— ●) and 6 untrained subjects (■ - - - - - - - ■). All subjects exercised for four successive periods of 8 min, each at 30%, 45%, 60% and 75% of their maximum capacity.


PLASMA HGH
μ U/ml
60
50
40
30
20
10
0
UNTRAINED
TRAINED
30%
45%
60%
75%
0
8
16
24
32
40
TIME - min

untrained subjects they remained elevated so that the values of HGH during and after exercise were significantly higher in the untrained subjects than the cyclists ($P < 0.01$).

DISCUSSION

There were considerable differences in the biochemical responses to exercise between the two groups. The trained cyclists had a significantly lower concentration of blood lactate and pyruvate. These changes persisted even when the trained subjects had worked harder in absolute terms than the control subjects as evidenced from the work loads. Thus these results are compatible with previous reports (Robinson and Harmon, 1941; Holmgren and Strom, 1959; Cobb and Johnson, 1963; Johnson et al., 1969). Metabolic mechanism may be adapted in athletes resulting in more efficient aerobic pathways. Evidence for this has been obtained in animals. Higher concentrations of certain oxidative enzymes have been reported after adaptation via training programme (Holloszy et al., 1971).

In these investigations there were also marked differences between the two groups on the effect of exercise on the blood concentrations of other metabolites.

The much greater rise in blood glycerol concentrations, in the trained cyclists, suggests that they have a great degree of fat mobilization. Both the concentrations of FFA and the rate of their increase with exercise were, however, lower in the cyclists and it therefore appears

that the cyclists utilise FFA liberated during exercise much more rapidly than the controls.

The rapid rise of blood glucose concentrations with exercise in the cyclists compared to the changes in the controls was remarkable and similar to the observation of Rennie et al., (1974). Working muscle at high work loads does not use circulating glucose as a fuel until muscle glycogen falls to a low level (Hultman, 1967; Wahren, 1970) and as reported above, FFA is probably metabolised to a greater extent by cyclists. It therefore appears that the large increase in blood glucose concentration was due to a decrease in the peripheral utilisation of glucose. This might be exaggerated by glucose entering the blood after stimulation of the liver by catecholamines and glucagon (Sutherland, 1950; Sutherland and Cori, 1951).

In this study there were also striking differences in the hormonal responses to the exercise between the two groups. Plasma IRI was depressed to a lesser extent in the cyclists during exercise and they also had a smaller increase in plasma IRI after the end of exercise. Normally, the major mechanism regulating insulin secretion from the pancreas is blood glucose concentration. Experiments using infusion techniques have produced evidence that adrenalin in animals (Kris et al., 1966) or noradrenaline in man (Porte and Williams, 1966) result in reduction or prevention of an increase in circulating insulin even after ingestion of glucose. During exercise there is known to be an increase in output of catecholamines (von Euler and Hellner, 1952; Vendsalu, 1960; Kotchen et al., 1971). Therefore, it seems reasonable to suppose that in both groups the failure of exercise induced hyperglycaemia to stimulate insulin

release could be due, at least in part, to an increased secretion of catecholamines. Lower concentrations of catecholamine metabolites in the urine of fit individuals compared with untrained subjects have been reported during exercise (von Euler and Hellner, 1952). The differences in adrenergic activity during exercise between the two groups may explain their differences in plasma IRI concentrations. It is also possible that increased aerobic oxidation of fat and lactate as a result of training may reduce the need for insulin in the cyclists as FFA and lactate transport across the cell membranes is independent of insulin (Williams and Ensinck, 1966).

This suggestion is consistent with the observation of Devlin (1963) who observed insulin-like activity (ILA) before and after a training programme in man and found that the fall of ILA during exercise was less after the programme.

During exercise plasma HGH concentrations were lower in the cyclists and they returned to resting values rapidly after the end of exercise. Similar observations related to physical fitness have been reported (Sutton et al., 1969). Although the role of HGH during exercise has not been clearly established, it has been suggested that it causes lipolysis and enhances the availability of FFA as a fuel (Hunter et al., 1965). The early changes in FFA concentrations are, however, probably due to adrenergic activity (Havel and Goldfien, 1959). The action of HGH is delayed (Raben and Hollenberg, 1959). However, HGH may enhance glucose utilisation soon after its release (Frohman et al., 1967).

The present results demonstrate significant differences in IRI and HGH responses to exercise between athletically trained and untrained subjects, even when the physically active groups worked at the same percentage of their maximum capacity as the untrained subjects.

As there are differences in the concentrations of IRI with exercise between the two groups, there could be differences in tissue sensitivity to insulin. Increased body weight as well as fat content is known to reduce the sensitivity of insulin in obesity (Rabinowitz and Zierler, 1962). Although no direct measurements have been made in this study to assess the degree of obesity, there is evidence to suggest that individuals who exercise regularly have a greater lean body mass and smaller fat tissue than sedentary individuals of the same weight (Parizkova, 1963; Björntorp et al., 1972). The cyclists were, however, lighter and nearer to their desirable weight than the controls and this may have resulted in higher insulin sensitivity in the peripheral tissue of the trained subjects.

SUMMARY

1. In order to obtain information on the range of hormonal and metabolic changes which occur in normal subjects, six well-trained subjects and six untrained subjects have been studied. They were investigated on two occasions. On the first occasion, the maximum work capacity for all the subjects was determined by increasing the work loads until exhaustion. On the second occasion the subject exercised for four successive periods for 8 minutes each at 30%, 45%, 60% and 75% of their maximum capacity. Venous blood samples were taken before, during and after exercise and were analysed for blood hormones and metabolites.

2. There were remarkable differences in the patterns of blood hormones and metabolite responses to exercise between the two groups. During exercise the fall in plasma IRI and the rise in plasma HGH were much less in the cyclists even when they exercised at similar percentages of their maximum capacity as the untrained subjects. The cyclists also had lower concentrations of blood lactate, pyruvate and FFA and higher concentrations of blood glucose and glycerol.

3. These observations indicate that physical training alters IRI and HGH values in relation to exercise. There may be not only changes in concentrations, but also changes in tissue sensitivity to IRI.

CHAPTER 2

STUDIES OF PATIENTS WITH OBESITY COMPARED WITH NORMAL SUBJECTS

INTRODUCTION

In the study reported in chapter 1 there were differences in metabolic responses to exercise between well trained cyclists and untrained subjects. The cyclists had lower blood concentrations of lactate, pyruvate, and FFA and higher concentrations of glucose and glycerol during exercise. Hormonal differences between the two groups were also observed both during and after exercise. These might be true metabolic differences between athletes and untrained subjects which could be due to adaptation as a result of athletic training. It was also suggested that there could have been differences in metabolism due to mild obesity, which was relatively greater in the untrained subjects (table 1.1).

Many metabolic and hormonal abnormalities have been reported in obesity. These have included an increase in total number of adipose tissue cells (Hirsch et al., 1966), increase in serum fatty acids (Opie and Walfish, 1963) and also in triglycerides (Albrink and Meigs, 1965). The fasting concentration of IRI is elevated (Bagdade et al., 1967), and although the secretion of IRI in response to glucose load is greater than in lean subjects (Karam et al., 1963), glucose tolerance may be decreased due to reduced peripheral uptake of glucose (Butterfield et al., 1965). A decreased HGH response to starvation and insulin induced hypoglycemia is also reported in obesity (Beck et al., 1964). These changes in obesity may result in abnormal energy metabolism during activity for although the rate of lipogenesis increased in obesity, the rate of fat mobilization appears to decrease in response to various stimuli such as starvation (Gordon, 1960), and

nor-adrenaline infusion (Ortho and Williams, 1960). Exercise is known to accelerate metabolic activity. This chapter reports a study of fat and carbohydrate metabolism during and after exercise together with studies of HGH and IRI made in patients with obesity and the results compared with normal controls. These investigations were carried out to provide further information about the metabolic effects of exercise. Such observations were necessary to give a background to studies reported later in this thesis upon the effects of drugs upon metabolism in obese subjects.

METHODS

Subjects:

Five patients (three female and two male) with obesity and six healthy male controls (table 2-1) were studied. No patient had undergone dietary restriction in the year before the study and were not receiving drugs at the time of the investigation. The obese subjects were 78% ($\pm$ 8) overweight. The control subjects were from the medical staff and research workers at the hospital. They were slightly overweight (13% $\pm$ 2) but none was known to have a metabolic disorder.

Procedure:

Patients and controls were brought to the laboratory after an overnight fast, and exercised for a period of 30 min on a bicycle ergometer fixed at a work load of 500 $\pm$ 100 Kpm/min. Heart rate was recorded during the investigation and for a further 15 min using an electrocardiograph. A catheter was placed in the antecubital vein and blood samples were taken before exercise, at 5 min intervals during

Table 2.1: Details of five patients with obesity and six normal controls

	Sex	Age (yr)	Height (cm)	Weight (Kg)	Reciprocal ponderal index ($ht/\sqrt[3]{wt}$)	% Increase above ideal weight
A. PATIENTS WITH OBESITY						
D.D.	F	51	159	112	33.14	102
M.McE.	F	47	158	91	35.07	65
E.H.	F	60	163	112	33.95	96
C.S.	M	48	171	122	34.58	68
R.D.	M	64	160	98	35.40	63
Mean		51	162	108	34.42	78
SEM		± 2	± 2	± 6	± 0.40	± 8
B. NORMAL SUBJECTS						
W.R.	M	31	168	75	39.63	18
M.J.	M	25	188	95	41.46	17
R.H.	M	39	173	72	41.82	6
D.P.	M	32	178	74	42.04	8
R.C.	M	25	172	74	41.40	10
S.S.	M	28	176	78	41.22	16
Mean		30	175	78	41.22	13
SEM		± 2	± 3	± 3	± 0.35	± 2

exercise and then at 15, 30, 60 and 90 min afterwards. The samples were treated as described in appendices I and II and analysed for lactate, pyruvate, glucose, glycerol, ketone-bodies, FFA, HGH and IRI.

RESULTS

Heart Rates (Fig 2:1):

The resting heart rates were 83 beats/min (± 5 SEM) in the patients and 77 beats/min (± 3 SEM) in the controls. In both groups heart rate increased during the first 15 min of exercise (controls 84%, patients 80%) after which the rate was steady until the end of exercise when a rapid fall occurred to within 11 - 16% of the resting heart rate 15 min after exercise. The percentage heart-rate changes in the patients and the controls were not significantly different at any time during exercise or the post-exercise period.

Pyruvate (Fig 2:2):

Resting pyruvate concentrations were similar in both groups. Blood pyruvate concentrations rose to a maximum at 15 min of exercise in the controls whereas in the patients the maximum occurred at the end of exercise. Pyruvate concentrations were relatively higher in the patients both during exercise and in the post-exercise period, but they were not significantly different. The concentrations then fell towards normal levels by the end of the investigation.

Lactate (Fig 2:2):

Resting concentrations of blood lactate were not significantly different between the two groups ($P > 0.05$). Rapid elevation of blood

Fig. 2.1. Heart rate (beats/min, mean ± SEM) in 6 control subjects (■ ------- ■) and 5 obese patients (● ——— ●) during and after 30 min of exercise.

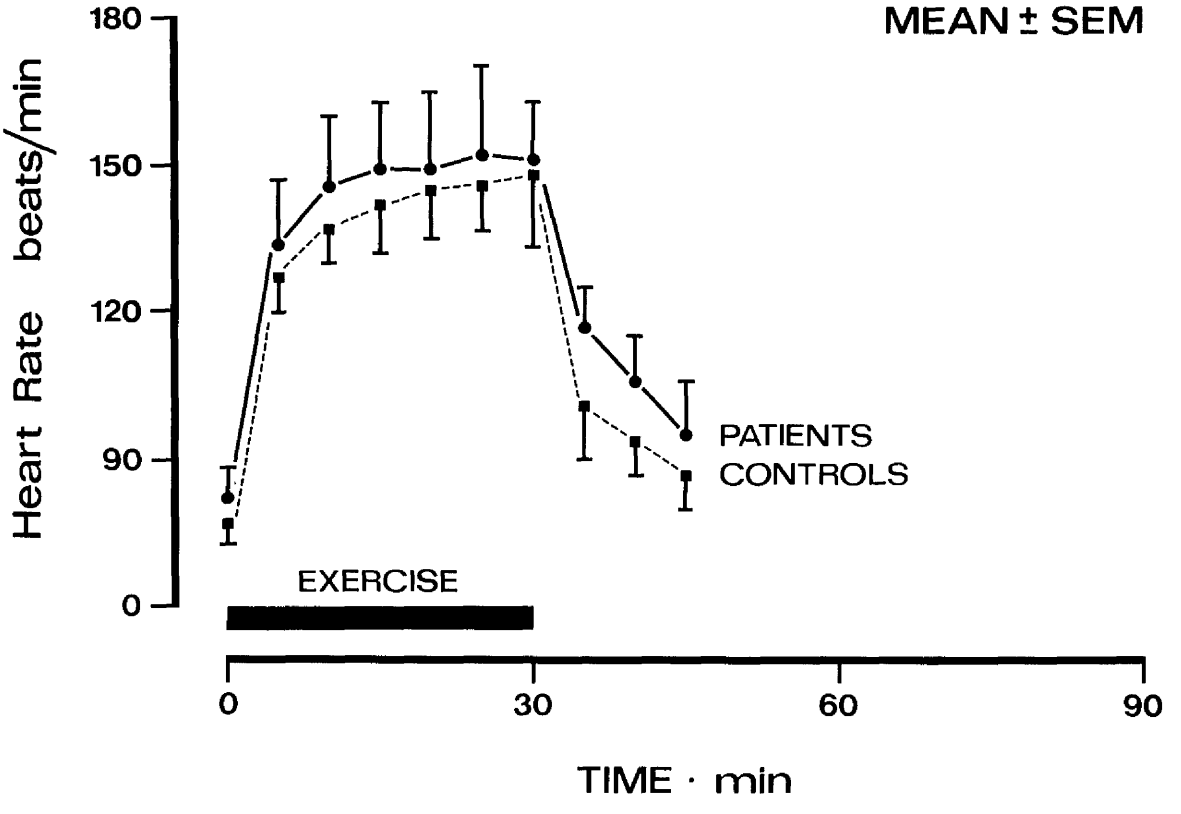
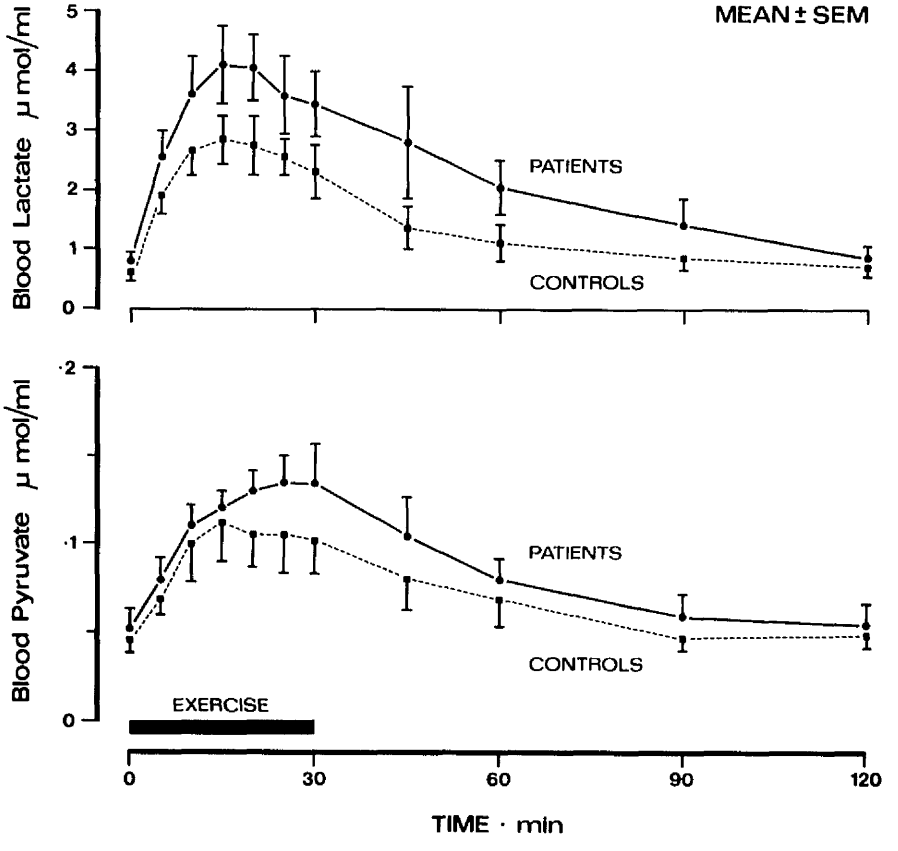

MEAN ± SEM
Heart Rate beats/min
180
150
120
90
0
PATIENTS
CONTROLS
EXERCISE
0
30
60
90
TIME · min

Fig. 2.2. Blood pyruvate and lactate (μmol/ml, mean ± SEM) in 6 control subjects (■ ------- ■) and 5 obese patients (● ——— ●) during and after 30 min of exercise.


MEAN ± SEM
Blood Lactate μ mol/ml
5
4
3
2
1
0
PATIENTS
CONTROLS
Blood Pyruvate μ mol/ml
·2
·1
0
PATIENTS
CONTROLS
EXERCISE
0
30
60
90
120
TIME · min

lactate values to a peak at 15 min of exercise occurred in both groups. The maximum being significantly greater in the patients. The concentrations in the patients remained significantly different ($P < 0.05$) from their resting values for the remaining part of exercise and for 30 min after the end of exercise. The controls, however, showed a more rapid return to approximately pre-exercise values so that there was no significant difference between the resting concentrations and those 30 min after the end of exercise.

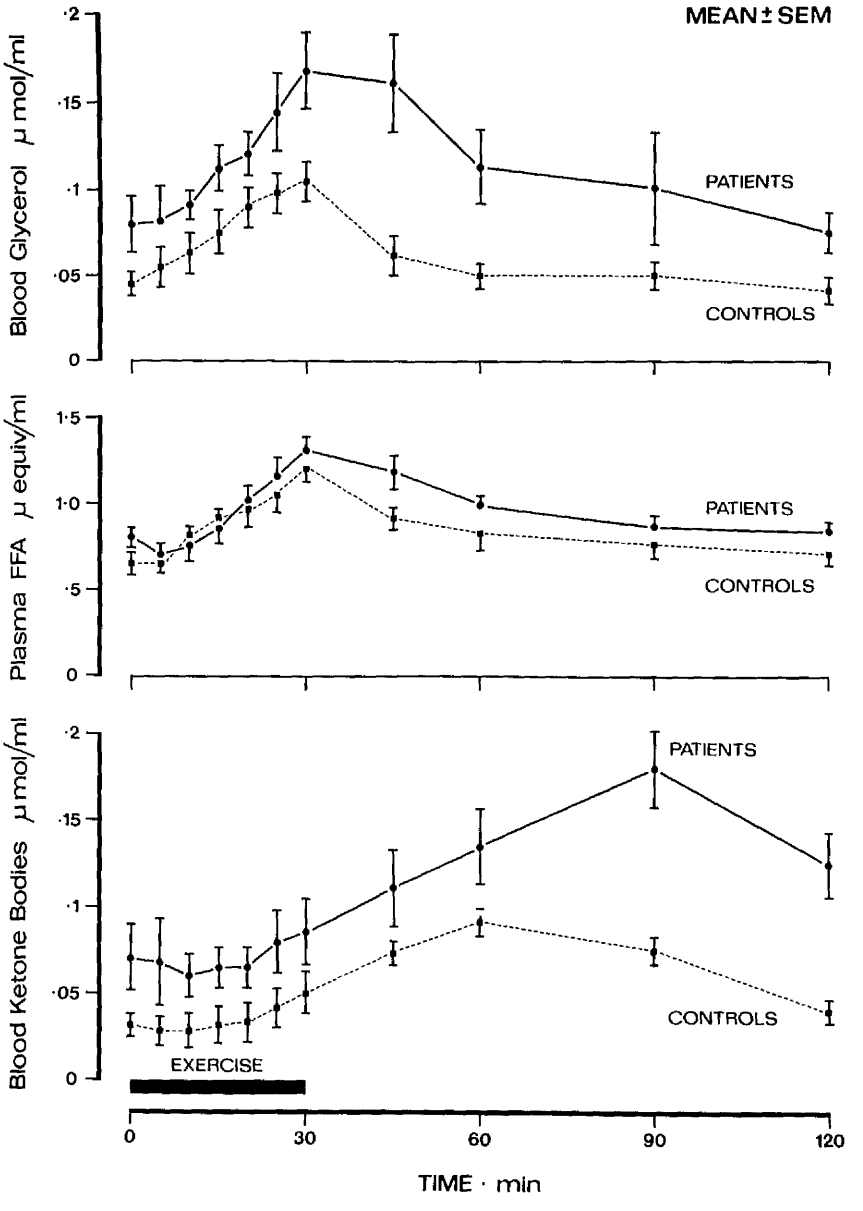
Glycerol (Fig 2.3):

Blood glycerol concentrations were significantly higher ($P < 0.05$) at rest in the patients compared with those of the controls. The peak values were significantly higher in the patients than of the controls, but the percentage increase (180%) was similar in both groups. The concentrations in the patients remained elevated 15 min after exercise and returned slowly to resting values at the end of the investigation. Glycerol concentrations in the controls, however, showed a rapid return towards resting values and at 15 min after exercise the values were not significantly different from those at rest.

Ketone-bodies (Fig 2.3):

Blood ketone-body concentrations (acetoacetate + 3 hydroxybutyrate) were significantly higher at rest in the patients compared with controls ($P < 0.01$). Concentrations at rest and at the end of 30 min of exercise were not significantly different in both groups. After exercise the concentrations increased considerably both in controls and in the patients, but the maximum values occurred in the obese patients 30 min after the maximum in the controls.

Fig. 2.3. Plasma FFA (μEquiv/ml), blood glycerol and ketone-bodies (μmol/ml, mean ± SEM) in 6 control subjects (■ -------- ■) and 5 obese patients (● ——— ●) during and after 30 min of exercise.


MEAN ± SEM
Blood Glycerol μ mol/ml
·2
·15
·1
·05
0
PATIENTS
CONTROLS
Plasma FFA μ equiv/ml
1·5
1·0
·5
0
PATIENTS
CONTROLS
Blood Ketone Bodies μ mol/ml
·2
·15
·1
·05
0
PATIENTS
CONTROLS
EXERCISE
0
30
60
90
120
TIME · min

FFA (Fig 2.3):

Resting plasma FFA concentrations were significantly higher ($p < 0.05$) in the patients than in controls. During exercise the concentrations rose in both the patients and controls to a maximum at the end of exercise when there was no significant difference between the two groups ($p > 0.05$). The patients showed a small fall in FFA concentration 5 min after the beginning of exercise. Although FFA concentrations were relatively higher in the patients both during the second half of exercise and in the post-exercise period, they were significantly different at only 15 min after exercise. On both occasions the concentrations returned to approximately pre-exercise values by the end of the investigation.

Glucose (Fig 2.4):

Resting blood glucose concentrations were not significantly different between the groups ($p > 0.05$). Exercise caused a fall in the concentrations earlier in the controls. The minimum occurred at 20 min in both controls and patients, and values increased both in the remaining period of exercise and for 30 min afterwards. Controls values were significantly lower throughout exercise and at 15 and 30 min afterwards.

Plasma HGH (Fig 2.5):

Plasma HGH levels were similar at rest in both groups. They rose by 600% in the controls and 350% in the patients by the end

Fig. 2.4. Blood glucose (mg/100 ml) and plasma IRI (μ units/ml, mean ± SEM) in 6 control subjects (■ ——— ■) and 5 obese patients (● ——— ●) during and after 30 min of exercise.

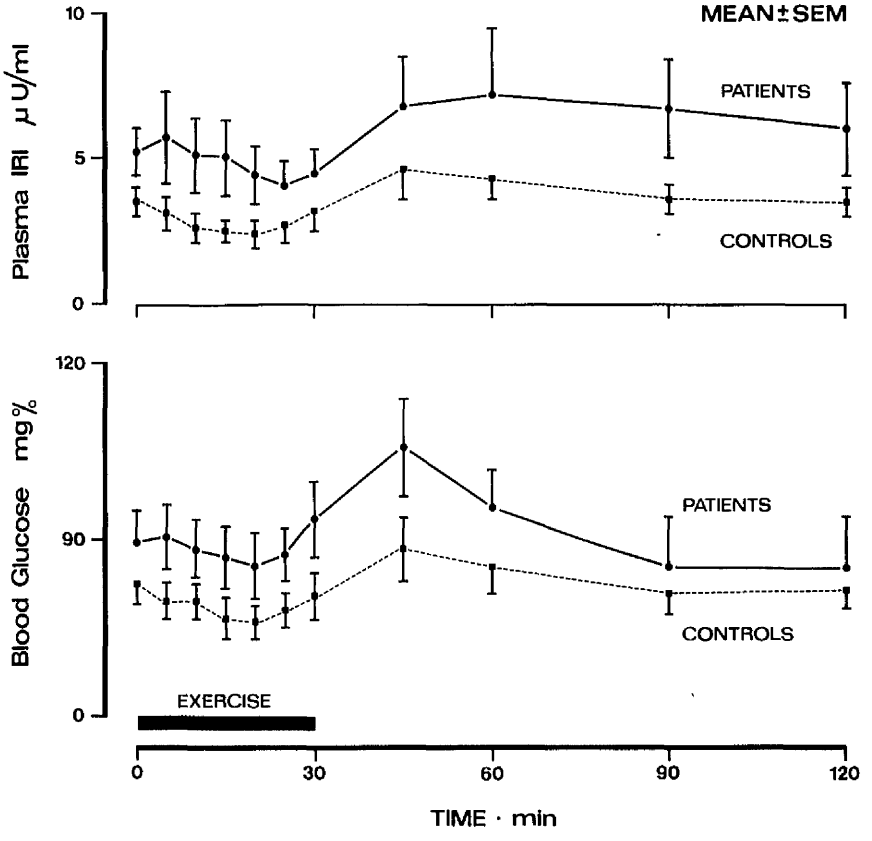
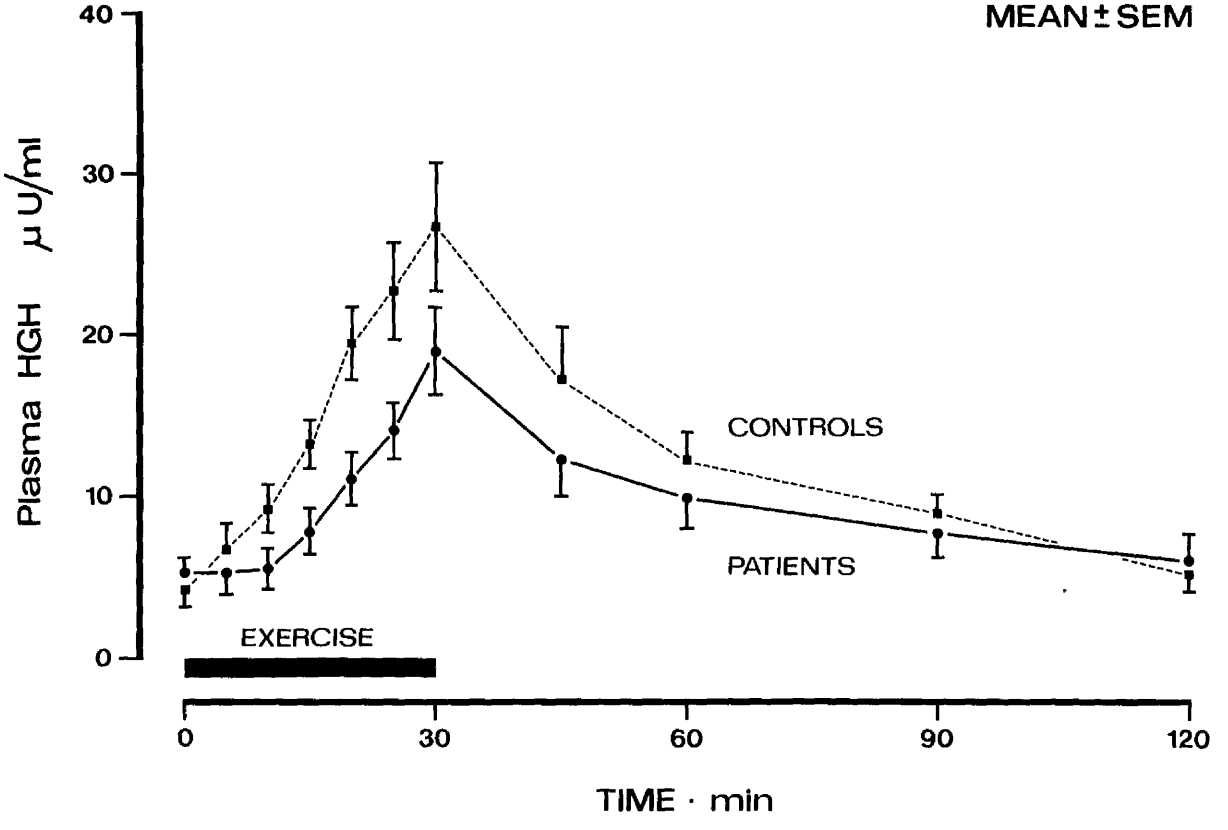

MEAN±SEM
Plasma IRI μU/ml
10
5
0
PATIENTS
CONTROLS
Blood Glucose mg%
120
90
0
PATIENTS
CONTROLS
EXERCISE
0
30
60
90
120
TIME · min

Fig. 2.5. Plasma HGH (μ units/ml, mean ± SEM) in 6 control subjects (■--------■) and 5 obese patients (●————●) during and after 30 min of exercise.


MEAN ± SEM
Plasma HGH μU/ml
40
30
20
10
0
CONTROLS
PATIENTS
EXERCISE
0
30
60
90
120
TIME · min

of exercise. The patients showed a small change in the first 10 min of exercise. The concentrations at 10, 15, 20, 25 and 30 min were significantly higher ($p < 0.01$) during exercise in the controls compared with the patients. In the 90 min following exercise HGH fell to resting levels in both controls and patients.

Plasma IRI (fig 2.4):

Resting concentrations of IRI were significantly higher in the patients than the controls ($p < 0.05$). The concentrations fell during exercise earlier in the controls, whereas in the patients they rose slightly in the early part of exercise and fell as the exercise continued. The minimum concentrations occurred at 25 min of exercise in both groups and values increased in both the remaining period of exercise and for 15 min afterwards. Although plasma IRI concentrations were higher in the patients both during exercise and in the post-exercise period, they were significantly different at only 5, 10, 15 and 20 min of exercise.

DISCUSSION

Exercise is known to cause elevation of both blood glycerol and plasma FFA levels in normal subjects. This probably occurs as a result of increased rate of fat mobilization, stimulated by increased adrenergic activity. In this study the concentrations of blood glycerol were higher in the patients with obesity both during and after exercise. This suggests that the patients had a greater degree of fat mobilization. The concentrations of plasma FFA at the end of exercise were, however,

similar in both groups, but the concentrations at rest were higher in the patients and had fallen in the early part of exercise. It therefore appears that patients with obesity have the ability to mobilize and utilize more fat than the controls. The patients also showed a greater blood glucose level throughout and this may be further evidence for the greater reliance on fat as a fuel for energy during exercise in obesity (Harr et al., 1969).

After exercise the concentrations of both FFA and glycerol showed a rapid return to resting values in the controls whereas in the patients they remained elevated. Similar results have been reported by Shwarz and co-workers (1969) who suggested that the patients might have been able to mobilize fat in excess of their needs. As the concentrations of blood ketone-bodies hardly altered during exercise, there is probably an equilibrium between production and utilization. After exercise, however, when muscular utilization of fuel decreases, there is a rise in the ketone-body concentrations (Johnson et al., 1969; Johnson and Walton, 1972). The much greater rise after exercise in the patients may reflect a decrease in their ability to oxidise acetyl CoA through the citric acid cycle. The inadequate carbohydrate utilization and also insulin resistance which have been reported in obesity, would also tend to increase ketone-body production. The patients also had increased concentrations of lactate and pyruvate. Increased rate of fat oxidation increases the level of acetyl CoA which in turn blocks pyruvate oxidation, resulting in the accumulation of both pyruvate and lactate (Garland et al., 1964).

In this study the fall in the concentrations of plasma IRI and the rise in the concentrations of HGH in response to exercise were much less in the patients than of the controls. This could be explained if the patients were more fit than the controls (Sutton et al., 1968), but it is unlikely since the concentrations of blood lactate during exercise and of ketone-bodies after exercise were higher in the patients. They might have been expected to be lower than those of the controls if the degree of fitness was the explanation. There was a relatively poor response of HGH to hypoglycaemia in the obese subjects, as previously reported (Beck et al., 1964). This may account for the differences of HGH response between the two groups, if the fall in blood glucose during exercise was the stimulus. Another possibility is that the patients had a higher rate of fat mobilization as indicated by higher glycerol concentrations. This might have caused a reduced response of HGH to exercise since it has been found that increased fat mobilization has an inhibitory effect on the release of HGH. This occurs in response to hypoglycaemia induced by insulin whereas decreased mobilization stimulates the release of HGH in response to hypoglycaemia (Quabbe et al., 1972) and to exercise (Hartog et al., 1967).

It can be concluded that exercise causes changes in blood metabolites of carbohydrate and fat in obese patients of a similar pattern to controls, although the changes tend to be more marked. The changes in the concentrations of HGH and IRI, however, are less marked in obese patients and are probably related to obesity rather than differences in physical fitness.

SUMMARY

1. Five patients with obesity and six healthy volunteers were studied before, during, and after 30 min of moderate steady exercise on a bicycle ergometer. Venous blood samples were taken for estimation of blood metabolites and hormones.

2. Exercise caused changes in blood metabolites in obese patients of a similar pattern to controls. Pyruvate, lactate and glycerol were considerably elevated and post-exercise ketosis developed. These changes were more marked in the patients. Throughout the test FFA and glucose concentrations were higher in the obese than in the normal subjects.

3. During exercise the rise in plasma HGH and the fall in plasma IRI were less marked in the patients.

4. The differences in blood glycerol, FFA, and ketone-bodies between the patients and controls, may suggest a great reliance on fat metabolism in the patients. They also suggest that the higher rate of fat mobilization in the patients may reduce the response of both HGH and IRI to exercise.

5. It is concluded that these changes were related to metabolic adaptation due to obesity rather than differences in physical fitness.

CHAPTER 3

PATIENTS WITH OBESITY DURING AND AFTER DIETARY RESTRICTION

INTRODUCTION

In the comparison of exercise by patients with obesity and by healthy controls reported in chapter 2 there were differences in metabolic responses between the two groups. The patient had higher blood concentrations of lactate, pyruvate and glycerol during exercise and higher concentrations of blood ketone-bodies in the post exercise period. A decreased elevation in the concentrations of HGH and a smaller depression in the concentrations of insulin were also observed in the obese subjects during exercise. The physical activity of the obese patients was limited and, therefore, it was suggested that the differences in physical fitness between the two groups could account for the differences in metabolism. A further possibility was that there was altered energy metabolism (Gordon et al., 1962) because of metabolic and endocrine adaptations to obesity (Sims and Horton, 1968). It was concluded that these changes were probably related to metabolic adaptation to obesity rather than to differences in physical fitness.

Resting observations have been made in obese patients during starvation therapy for 2-4 weeks and are reported in chapter 7. During starvation by patients with obesity the blood concentrations of glucose fell, whereas concentrations of FFA and ketone-bodies rose. A rise in the ratio of 3-hydroxybutyrate/acetoacetate was also observed. As there are changes in blood concentrations of metabolites, the patterns of utilization of metabolic fuels by active tissues including muscle are also probably altered as a result of dietary restriction. Recent work in man has demonstrated that metabolic pathways of fat and carbohydrate are altered during the adaptation to starvation (Cahill et al., 1966;

Owen et al., 1969). Although some alteration in metabolic and hormonal responses as a result of starvation have been recognised at rest, there is little information about changes in metabolites and hormones with starvation when related to exercise.

An opportunity to study the metabolic and hormonal changes during exercise is provided by the study of a group of patients undergoing starvation therapy for obesity. Observations in this situation are described in this chapter and indicate remarkable differences in the patterns of metabolites, which are discussed in relation to plasma HGH and IRI concentrations.

METHODS

Subjects

Six adult female patients (table 3.1) with obesity were studied on two occasions. On the first occasion they were in hospital undergoing starvation therapy. One patient (A.B.) was receiving a diet of 400 kcal/day and the remainder were receiving water with added vitamins. The subjects were studied on second occasion after they had left hospital and were eating normal diet with no restrictions, but had fasted overnight before the investigation. One patient (M.S.) was only studied while being starved and was not prepared to have a further investigation after discharge.

Procedure

The investigation had been explained to all the subjects and their consent had been obtained. On the day of the investigation the

Table 3.1: Details of six patients with obesity during and after therapeutic starvation

	STARVATION							AFTER STARVATION				
	Age	Height cm	Duration days	Weight kg	Reciprocal Ponderal Index	Fat Thickness mm	% Fat	Days After Starvation	Weight kg	Reciprocal Ponderal Index	Fat Thickness mm	% Fat
MS	58	156	16	99	33.76	135	41.0		-	-	-	-
BS	34	156	4	95	34.28	130	41.0	20	96	34.02	136	42.0
JN	44	168	5	126	26.40	145	42.0	16	128	25.64	140	42
AB	44	158	18	86	35.86	125	40.0	24	87	35.64	120	40
EP	65	146	3	115	28.40	160	44.0	14	114	28.07	156	44.0
EB	32	145	21	63.0	36.40	116	40.0	12	63	36.43	124	41.0
Mean	46	155	11	98	32.50	136	41.00	17	98	31.96	135	42.0
SEM	±5	±3	±3	±9	±1.68	±6	±0.60	±2	±11	±2.15	±6	±0.66

subjects came to the laboratory between 09.00 and 10.00 hours. A catheter was placed in the antecubital vein and a first resting sample was taken. A further resting sample was taken 10 min later. They then exercised on either a bicycle ergometer or a treadmill for 30 min, followed by a period of rest of 90 min. The work on the bicycle and on the treadmill was regulated according to the subjects ability to achieve a mean heart rate of 150 beats/min towards the end of exercise. Heart rate was recorded using an electrocardiograph. Blood samples were taken at 5 min intervals during exercise and then at 15, 30, 60 and 90 min afterwards. The blood samples were treated as described in appendices I and II and analysed for lactate, pyruvate, glucose, glycerol, ketone-bodies, FFA, HGH and IRI.

RESULTS

Heart Rate (fig.3.1) In all subjects similar heart rates were achieved at the end of exercise and the heart rates for the last 10 min of exercise were not significantly different on the two occasions ($P > 0.05$) although the rate of increase at the beginning of exercise was greater during starvation.

Glucose (fig 3.2) Blood glucose concentrations were significantly higher at rest in the control investigation compared with those during starvation. At the end of thirty min of exercise glucose concentrations had risen 16% from resting levels in the control investigation, but with starvation the concentrations showed a greater rise, 60% above resting levels, so that there was no significant difference at that stage between

Fig 3.1. Heart rate (beats/min, mean ± SEM) in 5 patients with obesity after two periods of 30 min exercise.

Starvation (●——●); normal diet (■ - - - - ■).

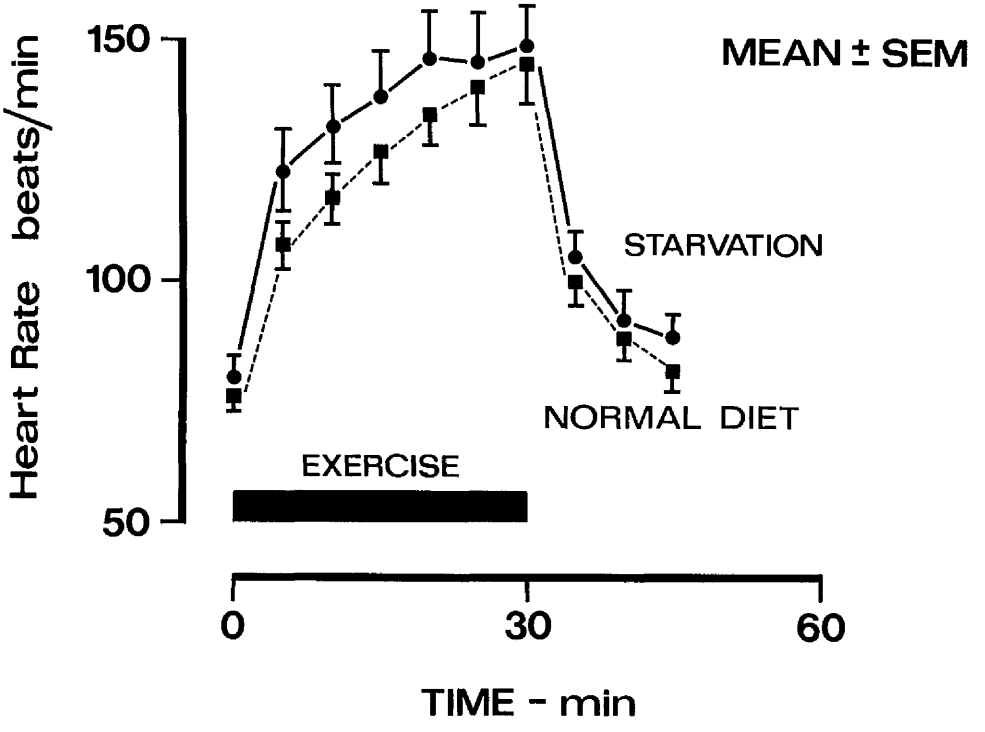
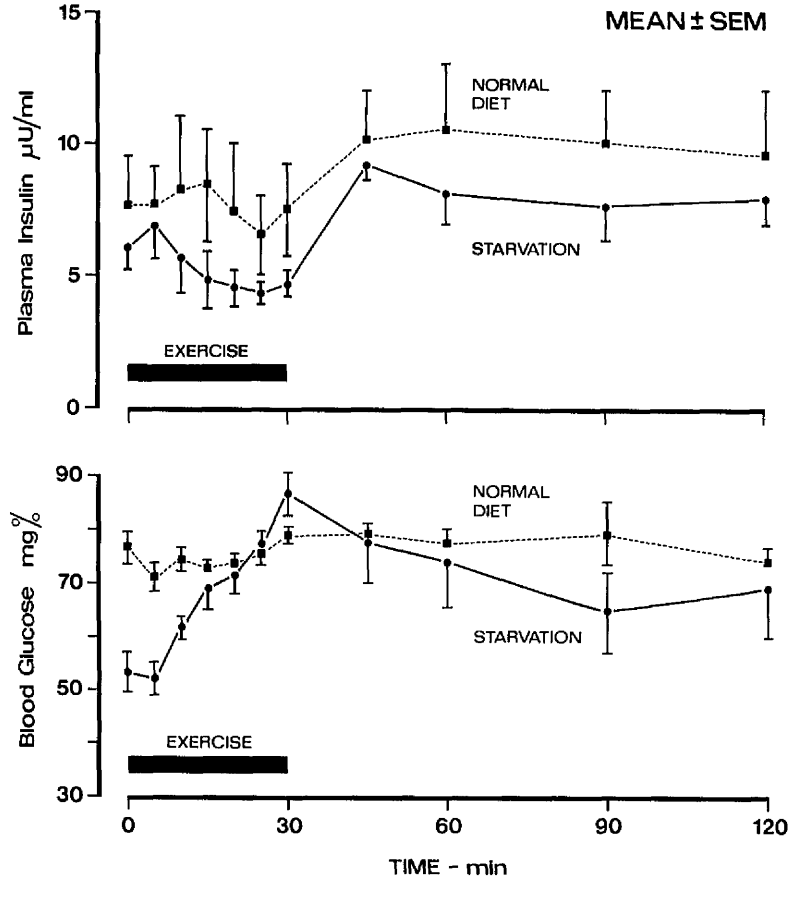

Heart Rate beats/min
150
100
50
MEAN ± SEM
STARVATION
NORMAL DIET
EXERCISE
0
30
60
TIME - min

Fig 3.2. Blood glucose (mg/100 ml) and plasma IRI (μ units/ml, mean ± SEM) in 5 patients with obesity after two periods of 30 min exercise.

Starvation (○ ——— ○); normal diet (■ ——— ■).


MEAN ± SEM
Plasma Insulin μU/ml
15
10
5
0
NORMAL DIET
STARVATION
EXERCISE
Blood Glucose mg%
90
70
50
30
NORMAL DIET
STARVATION
EXERCISE
0
30
60
90
120
TIME - min

the two occasions. During recovery glucose concentrations were relatively higher on the control occasion and they returned to resting levels 60 min after exercise, whereas with starvation the concentrations remained about 26% above the resting values for the remainder of the investigation.

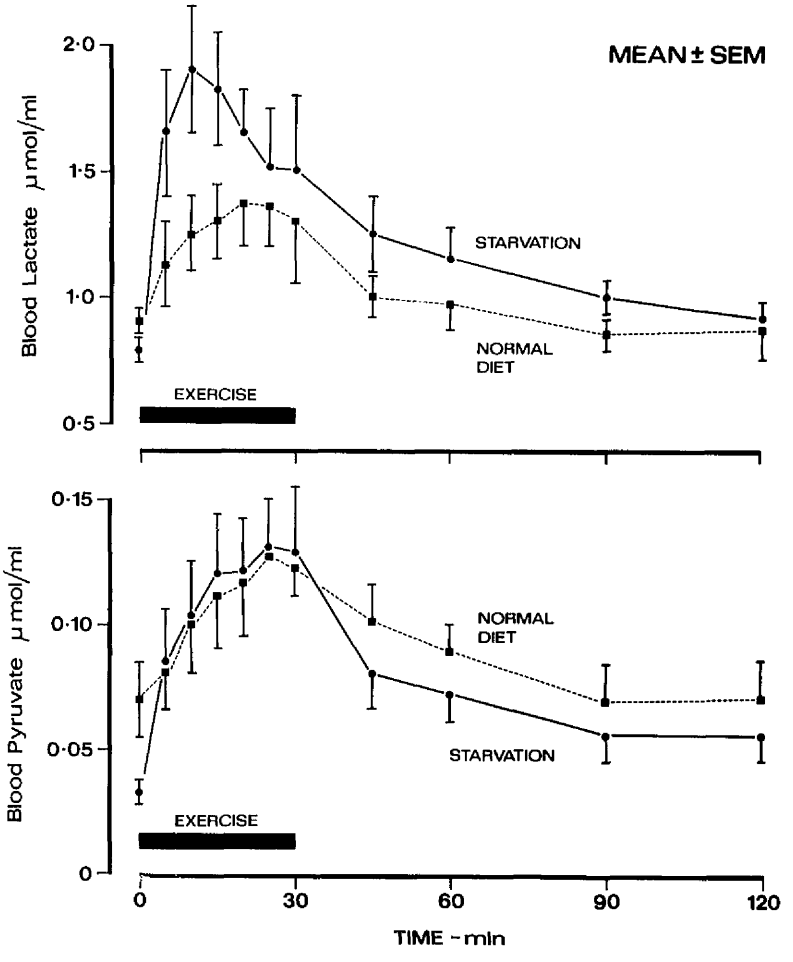
Lactate (fig 3.3) At rest lactate concentrations were significantly lower with starvation than on the control occasion. Rapid elevation of blood lactate values to a peak at 10 min of exercise occurred with starvation, whereas in the control investigation the peak occurred at 20 min. Although lactate concentrations were relatively higher with starvation both during exercise and in the post-exercise period, they were significantly different at only 5, 10 and 15 min during exercise and at 15 and 60 min after exercise. On both occasions lactate concentrations returned to resting levels by the end of the investigation.

Pyruvate (fig 3.3) Resting pyruvate concentrations were significantly ($p < 0.05$) lower with starvation than on the control occasion. During exercise the concentrations rose to a maximum at 25 min when there was no significant difference between the two occasions. Pyruvate concentrations returned to resting values 60 min after exercise in the control investigation, whereas with starvation the concentrations remained about 16% above the resting levels for the remainder of the investigation, but as the resting concentration during starvation was much lower the post-exercise levels were below the control values.

FFA (fig 3.4) With starvation FFA concentrations were significantly higher at rest before exercise than on the control occasion ($P < 0.01$)

Fig 3.3. Blood pyruvate and lactate (μmol/ml, mean ± SEM) in 5 patients with obesity after two periods of 30 min exercise.

Starvation (● ——— ●); normal diet (■ ——— ■).


MEAN ± SEM
Blood Lactate μmol/ml
2·0
1·5
1·0
0·5
STARVATION
NORMAL DIET
EXERCISE
Blood Pyruvate μmol/ml
0·15
0·10
0·05
0
NORMAL DIET
STARVATION
EXERCISE
0
30
60
90
120
TIME - min

Exercise caused a slight fall in concentrations on the control investigation (4% of resting), but with starvation the fall was greater (16% of resting). On both occasions FFA concentrations rose after the initial fall to maximum towards the end of exercise. The maximum value was higher with starvation, but the percentage rise was greater on the control occasion (42% of resting) than with starvation (13% of resting). On the control occasion FFA concentrations returned to resting levels by the end of the investigation, whereas with starvation FFA remained elevated in the post-exercise period.

Glycerol (fig 3.4) Resting glycerol concentrations were significantly higher with starvation than in the control investigation ($p < 0.01$). Blood glycerol concentrations increased during exercise earlier on the control occasion. The peak values were not significantly different, but they occurred at different times. With starvation the peak was at 25 min of exercise, whereas in the control investigation it was at the end of exercise. The concentrations at 5, 10 and 15 min were significantly ($p < 0.05$) higher during exercise on the control occasion than with starvation. On both occasions glycerol concentrations returned to resting levels by the end of the investigation.

Ketone-bodies (fig 3.5) At rest, blood ketone-bodies (3-hydroxy-butyrate + acetoacetate) concentration was 0.060 μmol/ml ($\pm$ 0.02 SEM) on the control occasion and 1.5 μmol/ml ($\pm$ 0.2 SEM) with starvation. On the control occasion, exercise caused a slight fall in concentrations for 15 min (14% of resting) but with starvation the fall was more marked (45% of resting) and the maximum fall was observed at 20 min. On both occasions the levels rose after the initial fall both

Fig 3.4. Plasma FFA (μequiv/ml) and blood glycerol (μmol/ml, mean ± SEM) in 5 patients with obesity after two periods of 30 min exercise.

Starvation (● ——— ●); normal diet (■ ——— ■).

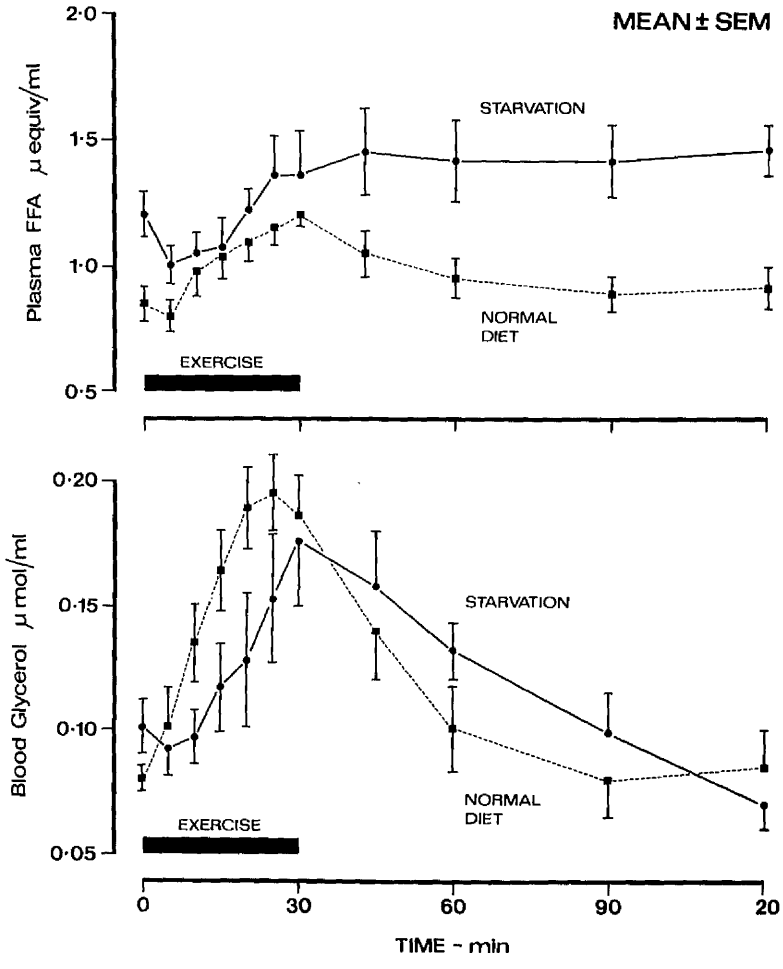

MEAN ± SEM
Plasma FFA μ equiv/ml
2·0
1·5
1·0
0·5
STARVATION
NORMAL DIET
EXERCISE
Blood Glycerol μ mol/ml
0·20
0·15
0·10
0·05
STARVATION
NORMAL DIET
EXERCISE
0
30
60
90
20
TIME - min

Fig 3.5. Blood ketone bodies (μmol/ml, mean ± SEM) in 5 patients with obesity after two periods of 30 min exercise.

Starvation (●——○); normal diet (■ - - - - - - - ■).

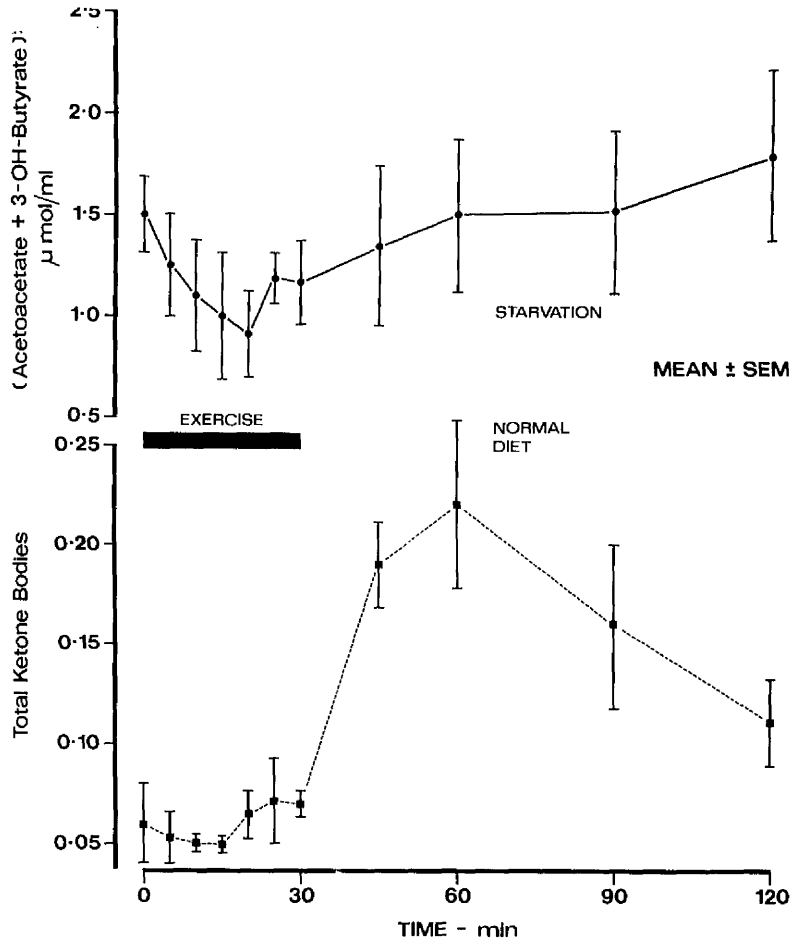
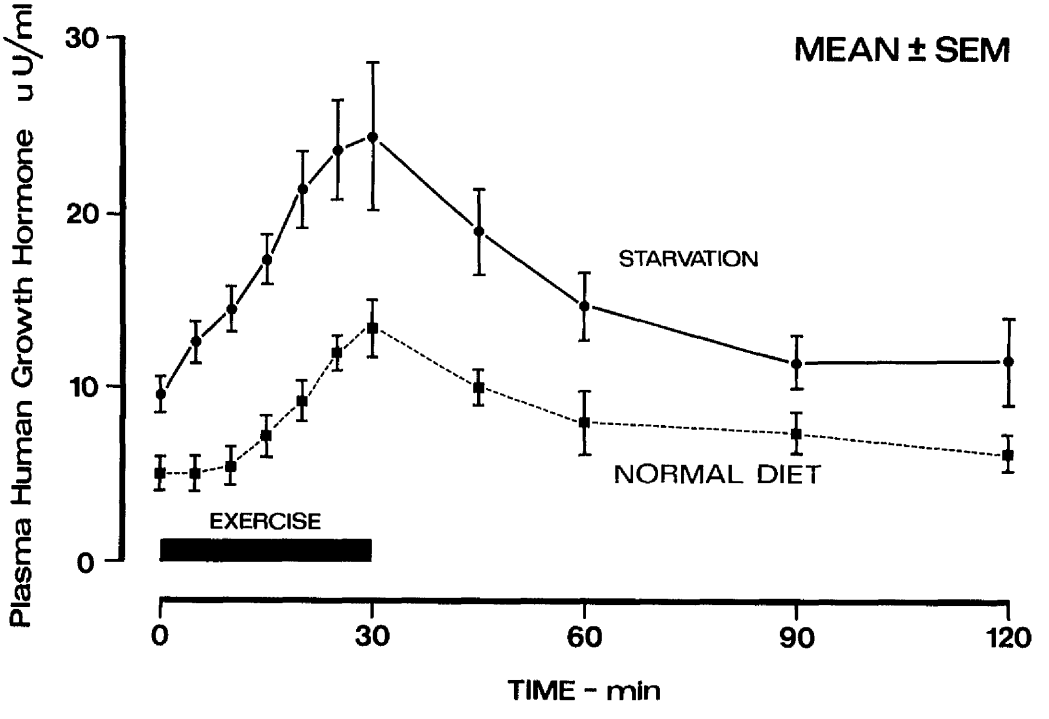

(Acetoacetate + 3-OH-Butyrate)
μ mol/ml
2·5
2·0
1·5
1·0
0·5
STARVATION
MEAN ± SEM
EXERCISE
NORMAL
DIET
Total Ketone Bodies
0·25
0·20
0·15
0·10
0·05
0
30
60
90
120
TIME - min

Fig 3.6. Plasma HGH (μ units/ml, mean ± SEM) in 5 patients with obesity after two periods of 30 min exercise.

Starvation (⊙ ——— ⊙); normal diet (▣ ------- ▣).


Plasma Human Growth Hormone u U/ml
30
20
10
0
MEAN ± SEM
STARVATION
NORMAL DIET
EXERCISE
0
30
60
90
120
TIME - min

in the remaining part of exercise and for 30 min afterwards. The concentration was much greater at 30 min after exercise on the control investigation (330% of resting) than with starvation (28% of resting).

Plasma HGH (fig 3.6) With starvation HGH concentrations were significantly higher before exercise than on the control occasion ($p < 0.01$). On both occasions exercise caused a rise in the concentrations to maximum at 30 min of exercise. With starvation the changes in absolute values were much greater (up to 15 μU/ml mean increase) than on the control occasion (up to 7 μU/ml mean increase). In the recovery period the concentrations of HGH returned to approximately pre-exercise values at 90 min after the end of exercise in both with starvation and control investigations.

Plasma IRI (fig 3.2) Resting plasma IRI concentrations were not significantly different between the two occasions; they rose slightly in the early part of exercise and fell as the exercise continued. The minimum concentrations occurred at 25 min on both occasions and values increased in both the remaining period of exercise and for 15 min afterwards. Control values were higher throughout exercise and during recovery but the differences in concentrations of IRI between the occasions were significant at only 15, 20 and 30 min of exercise.

DISCUSSION

With starvation, the subjects had higher concentrations of FFA, glycerol and ketone-bodies and lower concentrations of blood glucose

than on the control occasion. Thus these results are compatible with previous reports (Cahill et al., 1966; Owen et al., 1969).

Sympathetic activity is probably increased during starvation resulting in fat mobilization. The high concentrations of HGH together with low concentrations of IRI may also have contributed to the observed changes. The high concentrations of blood ketone-bodies during starvation were probably related to the concentrations of plasma FFA as the rate of formation of ketone-bodies depends on FFA concentrations (Wieland, 1965; Krebs, 1966).

In this study exercise caused an initial fall in the concentrations of FFA both when the subjects were starving and on the control occasion. This may be explained by increased FFA uptake by working muscle via increased peripheral blood circulation at the beginning of exercise. With starvation, the fall in the concentrations of FFA was more sustained and the percentage rise at the end of exercise was much less than on the control occasion, suggesting that during starvation the subjects are able to utilize more fat during exercise. Utilization of ketone-bodies by skeletal muscle has been demonstrated in animals (Blixenkrone-Möller, 1938) and in man (Hagenfeldt and Wahren, 1968). In the present studies exercise caused a fall in the concentrations of blood ketone-bodies on both occasions and this may have been due to increased rate of utilization. The change was greater with starvation, but the enzymes capable of utilizing ketone-bodies are widely spread among other tissues such as kidney, heart and brain (Medes et al., 1946; Williamson and Krebs, 1961; and Owen et al., 1967). It is likely therefore that the utilization of ketone-bodies by these organs is also

increased under the influence of starvation.

The rapid rise of blood glucose concentrations with exercise when the subjects were starving compared to the changes on the control occasion was remarkable and compatible with the observations of Hagenfeldt and Wahren (1971). Since all the subjects were overweight, it is possible that there was a decrease in the peripheral uptake of glucose and its subsequent oxidation (Butterfield et al., 1965). This could have been exaggerated by both increased fat and ketone-body utilization and also active glucose production by the liver during starvation. The rise of blood lactate and pyruvate in response to exercise was greater with starvation than in the control study. This is unlikely to be related to the concentration of glycogen in the exercising muscle since diminished glycogen stores of muscle have been found after a short period of starvation (Hultman and Bergstrom, 1967). It is possible that ketone-bodies may facilitate an aerobic glycolysis and lactate production by decreasing the intracellular oxidation of glucose (Williamson and Krebs, 1961). Blood levels of amino acids including alanine have been reported to increase during exercise by normal subjects (Felig and Wahren, 1970) and since starvation stimulates alanine uptake (Felig et al., 1969) by the liver, it is likely that alanine may be converted to pyruvate and later to lactate.

There were also differences in the hormonal responses to the exercise between the two occasions. The patients were able to raise their plasma HGH considerably during exercise on both occasions. The concentrations of plasma HGH were higher, however, at rest with starvation. They also rose to a greater extent during exercise with starvation compared

with the control occasion. This could have been due to a greater stress of exercise during starvation as was suggested by the more rapid rise of heart rate on that occasion. Protein catabolism, which is increased during starvation, may have also contributed to the greater rate of increases in the levels of HGH with starvation as this is a known stimulator of HGH release (Rabinowitz et al., 1968).

SUMMARY

1. Six adult female patients with obesity were studied before, during and after 30 min of moderate exercise on two separate occasions. On the first occasion they were in hospital undergoing starvation therapy. The subjects were also studied on another occasion after they had left hospital and were eating a normal diet. Heart rate was recorded and venous blood samples were taken for the estimation of metabolites and hormones.

2. Exercise caused a rise in the heart rates to a similar value at the end of exercise on both occasions, but the rate of increase at the beginning of exercise was greater during starvation.

3. During starvation the concentrations of pyruvate, lactate and glucose were lower at rest and rose during exercise to much greater extent than on the control occasion. The rise of blood glucose with starvation was 60%, whereas in the control study the rise was only 16% of resting.

4. Concentrations of FFA were higher at rest with starvation, fell in the early part of exercise and rose as the exercise continued. With starvation the fall was greater (16% of resting) compared with the control study (4%). The rise was smaller with starvation (13%) compared with the control findings (42% of resting).

5. In the control study there was a fall in the concentrations of ketone-bodies during exercise and a rise in the post exercise period, but with starvation the concentrations were considerably higher at rest and showed a remarkable fall with no further rise in the post exercise period.

6. Plasma HGH concentrations were higher at rest with starvation and rose during exercise to a much greater extent than on the control occasion.

7. IT IS CONCLUDED that exercise during starvation causes remarkable differences in metabolites, particularly FFA and ketones, and also hormonal concentrations in the blood compared with control observations.

These studies of exercise in starvation indicate that the sensitivity of the growth hormone release mechanisms are greatly increased in starvation. In addition support is given for previous observations that fat is the major fuel during starvation. These investigations also indicate that ketone-bodies are a major fuel in starvation contrary to some other reported studies.

CHAPTER 4

THE EFFECT OF ACETOACETATE UPON HUMAN GROWTH HORMONE AND INSULIN RELEASE

INTRODUCTION

When the blood levels of insulin and growth hormone were studied in trained cyclists and non-cyclists during and after cycling on a bicycle ergometer for 32 min, the cyclists showed a slight fall in the concentrations of IRI during exercise, but hardly any rise in IRI concentrations in the post-exercise period. The non-cyclists, however, showed a greater fall during exercise and a considerable rise in IRI concentrations after exercise, observations previously reported in chapter 1. A rise in the concentrations of ketone-body is known to occur in the post-exercise period and subjects in regular athletic training have a smaller rise compared with non-athletes (Johnson et al., 1969). The differences in ketone-body production and IRI release in athletes compared with non-athletes suggest that there is a relationship between ketosis and insulin release. The literature on this problem is confusing. In dogs increased level of IRI after ketone administration has been shown by many workers (Madison et al., 1964; Xavier et al., 1970). However, others have suggested that in man, the effect of ketones of lowering glucose and FFA is secondary to the inhibition of both fat mobilization from adipose tissue and glucose output from the liver (Balasse and Ooms, 1968; Senior and Loridan, 1968). To investigate the effect of ketone-body on the release of IRI, I therefore studied six subjects during an oral acetoacetate tolerance test.

METHODS

Subjects:

Six subjects, five males and one female (aged 30-56 yr) were studied. None of the subjects was known to have abnormal fat and

carbohydrate metabolism and they were eating normal diets with no restriction.

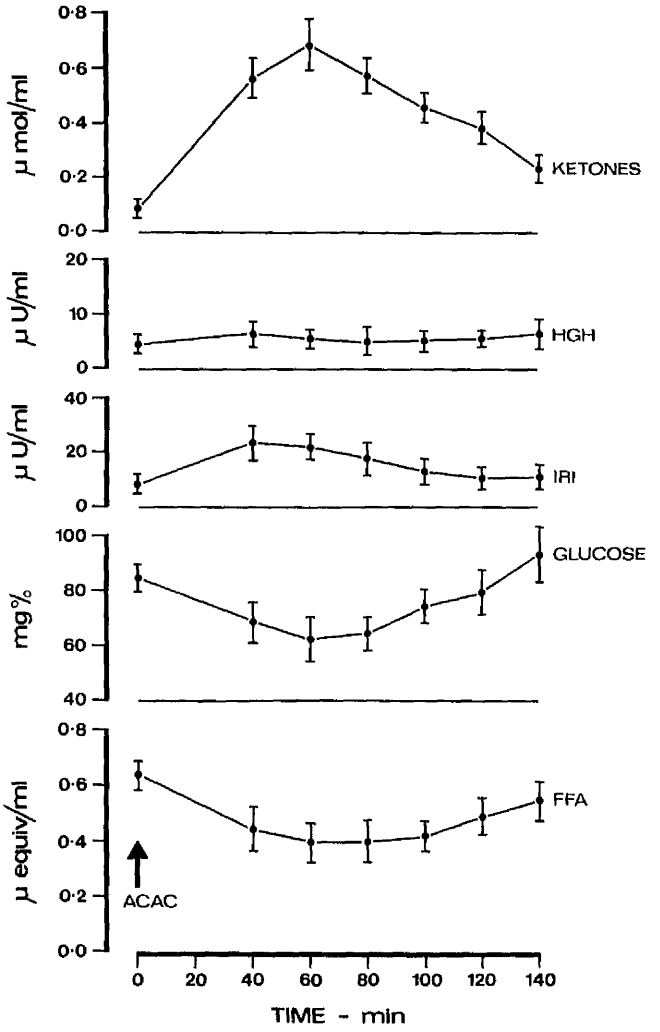
Procedure:

The subjects were brought to the laboratory after an overnight fast and remained at rest throughout the investigation, which was carried out between 0800 hr and 1000 hr. After catheterization, venous blood samples were taken before the ingestion of sodium acetoacetate (200 ml, 0.4M) (see page 18), 40 min later and then at 20 min intervals for a further 80 min. The blood samples were treated as described in appendices I and II and analysed for glucose, acetoacetate, 3-hydroxybutyrate, FFA, HGH and IRI.

RESULTS

The ingestion of sodium acetoacetate produced a peak level of total blood ketone-bodies of between 0.5 and 0.9 μmol/ml at 60 min and thereafter the blood level declined towards pre-ingestion value (Fig 4.1). Blood glucose fell from a fasting value of 85 mg% (± 5mg SEM) to 62 mg% (± 8 mg SEM) at 60 min and returned to pre-test value during the remaining period of the investigation. The concentrations at 60 and 80 min were significantly lower ($p < 0.01$) than fasting concentration. Plasma IRI rose from 8 μU/ml (± 3 μU SEM) to 23 μU/ml (± 6 μU SEM) at 40 min after the ingestion of acetoacetate, followed by a graaual decline to pre-ingestion value by the end of the investigation. The values at 40 and 60 min were significantly higher ($p < 0.05$) than fasting concentration. FFA concentrations fell promptly and reached a nadir at 60 min after the ingestion of acetoacetate. The concentration then returned towards the

Fig (4.1): The changes (mean±SEM) in the five subjects of total ketone-bodies (acetoacetate plus 3-hydroxybutyrate), HGH, IRI, glucose and FFA after ingestion of a solution of sodium acetoacetate (200 ml; 0.4M) at the time indicated.


0·8
0·6
0·4
0·2
0·0
μ mol/ml
KETONES
20
10
0
μ U/ml
HGH
40
20
0
μ U/ml
IRI
100
80
60
40
mg%
GLUCOSE
0·8
0·6
0·4
0·2
0·0
μ equiv/ml
FFA
ACAC
0
20
40
60
80
100
120
140
TIME - min

pre-test value by the end of the investigation. The values at 40, 60, 80 and 100 min were significantly lower ($p < 0.05$) than the pre-ingestion value.

DISCUSSION

Whereas the glucose lowering effect of ketone-bodies in dogs and in man has been known for many years (Neptune, 1956; Jenkins, 1967), the mechanism of the hypoglycaemia remains unclear. Mebane and Madison (1964) have reported that in dogs the hypoglycaemia results from a greatly decreased hepatic glucose output. They also have demonstrated that infusion of ketones was associated with a rise in insulin concentration in the pancreaticoduodenal vein (Madison et al., 1964). The present study shows that in man a significant enhancement occurred in the peripheral concentration of insulin with the ingestion of 0.4M acetoacetate. Insulin rose to a peak at 60 min after the ingestion of acetoacetate. This response was paralleled by those of ketones, suggesting a close relationship between the two. Subsequently, the fall in insulin concentrations coincided with the disappearance of the ketone-bodies. This finding is compatible with the observations reported by Xavier et al., (1970) and consistent with studies of isolated perfused pancreas which indicate that the secretion from this organ stops once the stimulatory substances disappeared (Grodsky et al., 1967). The rise in insulin concentrations obtained in this study may be sufficient to explain the observed fall in blood glucose. The FFA concentrations were also lowered after the ingestion of acetoacetate. This may be due to both increased insulin secretion and the direct effect of ketone bodies inhibiting FFA release (Björntorp and Scherston, 1967).

The degree of ketosis obtained in this study is within the range which may be observed in exercise, nevertheless no significant change in growth hormone occurred even though there were changes in glucose, insulin and FFA as a result of the ketone ingestion. When blood ketone bodies are raised during exercise, an increase in insulin secretion might be expected. Observations supporting this possibility are to be reported in the next chapter. It is therefore possible that ketone-bodies by stimulating the secretion of insulin, lower the blood concentrations of FFA and limit their production.

SUMMARY

1. In order to investigate the relationship between ketone bodies and hormone release, six subjects, five male and one female (aged 30-56 yr) were studied after the ingestion of acetoacetate. Venous blood samples were taken for the estimation of metabolites and hormones.

2. The ingestion of acetoacetate caused a rise in the concentrations of IRI and a fall in the concentrations of FFA and glucose. Acetoacetate however caused hardly any change in the concentrations of HGH. Those observations indicate that the degree of post-exercise ketosis may depend on differences in growth hormone secretion rather than vice versa.

3. It is also suggested that the rate of production of ketone bodies may be limited by a feedback mechanism dependent upon insulin release.

CHAPTER 5

AN EXAMINATION OF THE RELATIONSHIP BETWEEN KETONE-BODY CONCENTRATIONS AND INSULIN VALUES IN PATIENTS WITH ACROMEGALY

INTRODUCTION

In the previous chapter the following findings were reported: a fall in plasma FFA and blood glucose concentrations after the ingestion of acetoacetate was associated with a rise in concentrations of plasma IRI. These results indicated that fat mobilization is reduced by the presence of ketones. They also suggest that in man, ketone-body production may be limited by feed-back control dependent on insulin release. An opportunity to examine further the relationship between ketone-bodies and insulin release is provided by the study of patients with acromegaly. Acromegaly is an endocrinc disorder of the pituitary associated with an eosinophil tumour of the gland in which there is abnormally high production of HGH which not only affects bone growth but also metabolism (Martin, 1973). Patients with acromegaly show an abnormal peak in blood ketone-bodies and insulin during exercise and hardly any post-exercise ketosis (Johnson et al., 1971). These changes might have been due to the effect of the chronic elevation of HGH in the plasma as this hormone is known to reduce tissue sensitivity to insulin and to activate fat re-esterification (Merimee and Rabin, 1973). In normal subjects a second period of exercise carried out within several hours of the first results in a greater degree of post-exercise ketosis after the second exercise compared with that observed after the first exercise period. Studies of two periods of exercise should therefore provide an opportunity to examine the relationship of different concentrations of ketone-bodies and insulin in the same patient. Two patients with acromegaly have been studied and the results compared with three normal subjects.

METHODS

Subjects: two female patients (aged 24 and 57 yr) with acromegaly and three controls (one female and two male, aged 25 and 40 yr) were studied. The patients had clinical symptoms of a pituitary tumour and acromegaly. The control subjects were matched as far as possible for height and weight (table 5.1) and did not have a metabolic disorder. The acromegalic patients were investigated before therapy was instituted and they were not receiving drugs at the time of the investigation. All subjects were eating normal diets with no restriction.

Procedure: patients and controls were brought to the laboratory between 09.00 and 10.00 hours after overnight fasting. The subjects were exercised for two periods of 30 min with an interval of 90 min, on a bicycle ergometer fixed at a work load of 500 $\pm$ 100 kpm/min. The observations were made for a further 90 min after the second period of exercise. Venous blood samples were taken before exercise, at 5 min intervals during each period of exercise and then at 30, 60 and 90 min afterwards. Heart rate was measured and the blood samples taken and treated as described in appendices I and II. The samples were analysed for ketone-bodies and plasma IRI.

RESULTS

Ketone-bodies (Fig. 5.1): Resting concentrations of ketone-bodies (acetoacetate + 3-hydroxybutyrate) were similar in both groups, but changes during and after the two periods of exercise were different. After a decrease in the first 10 min the values in the patients rose

Table 5.1 : Individual data on age, weight, height and plasma HGH

Patients	Sex	Age yr	Weight kg	Height cm	Plasma HGH µU/ml		
					Rest	End of exercise	
						1st period	2nd period
A.T.	F	57	68	163	116	305	348
J.B.	F	24	65	160	68	94	116
controls							
M.J.	M	26	88	182	3.0	28	36
R.H.	M	39	73	173	2.1	25	38
M.S.	F	33	68	163	2.4	33	45

Fig (5.1) Changes in blood ketone-bodies (u mol/ml) in two acromegalic patients (▣——▣, □——□) and three control subjects (o——o) during and after two 30 min of exercise.

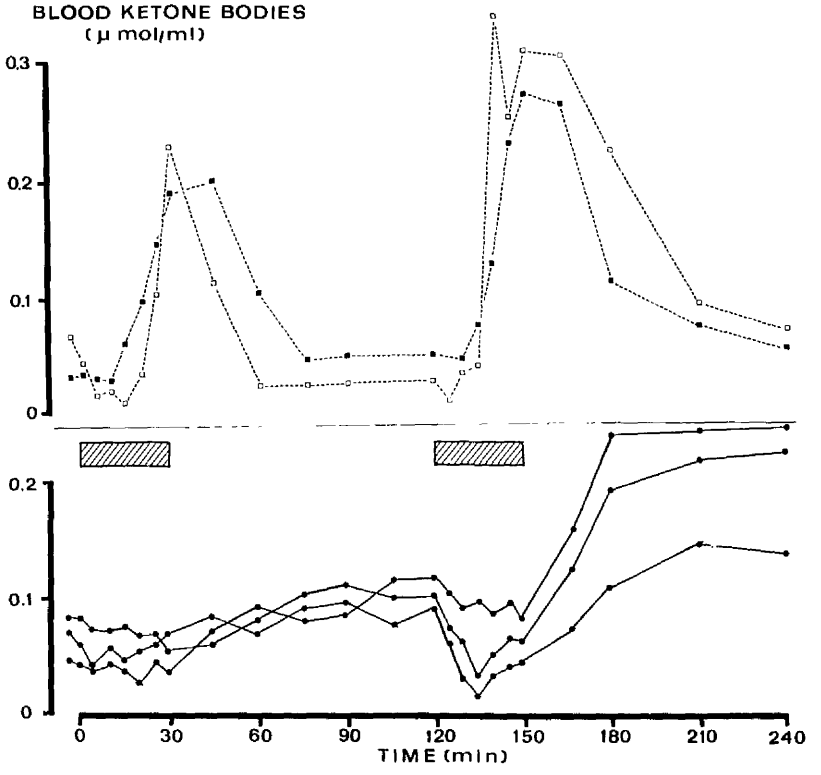

BLOOD KETONE BODIES
(μ mol/ml)
0.3
0.2
0.1
0
0.2
0.1
0
0
30
60
90
120
150
180
210
240
TIME (min)

Fig (5.2) Changes in plasma IRI (u units/ml) in two acromegalic patients (▣-----▣, □-----□) and three control subjects (⊙———⊙) during and after two 30 min of exercise.

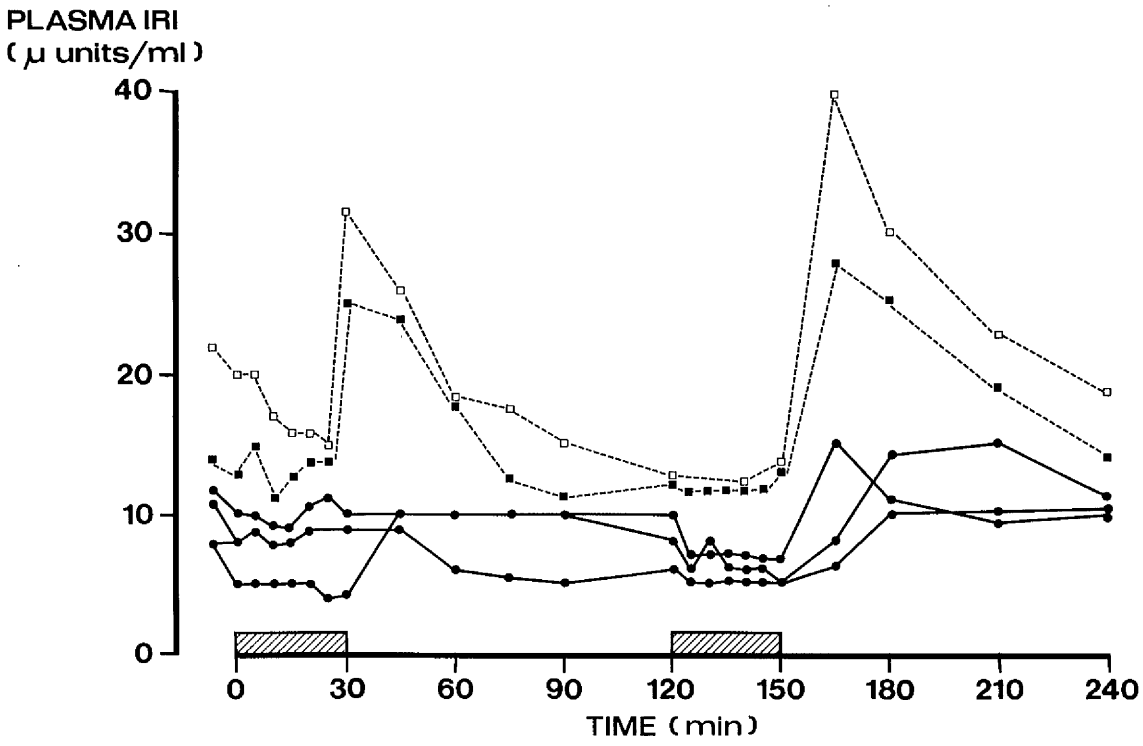
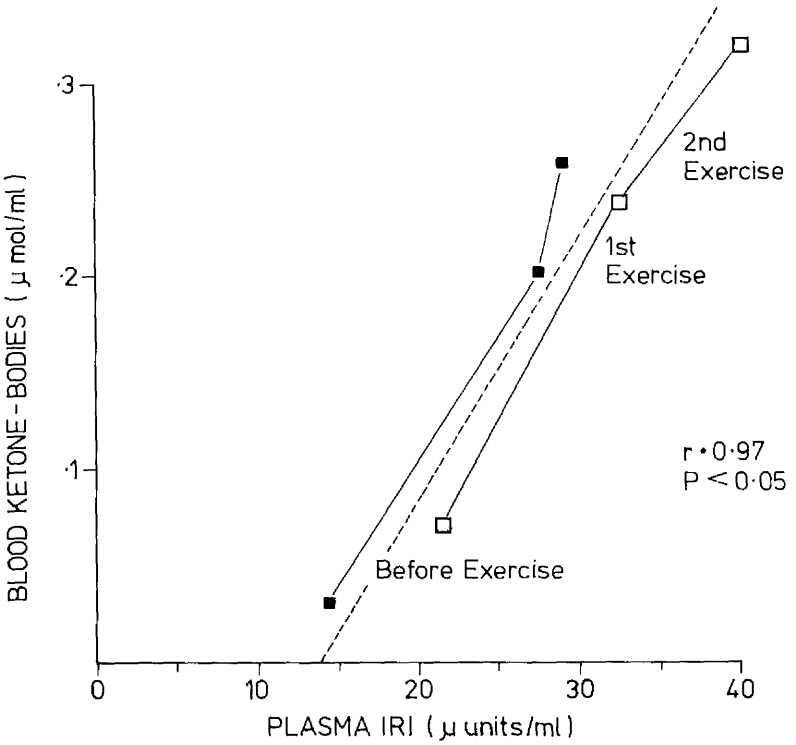

PLASMA IRI
(μ units/ml)
40
30
20
10
0
0
30
60
90
120
150
180
210
240
TIME (min)

Fig (5.3): The relationship between the highest observed values for total blood ketone-bodies and plasma insulin concentrations during exercise by patients with acromegaly. ($r.97$; $p < 0.05$).


·3
·2
·1
BLOOD KETONE-BODIES (μ mol/ml)
2nd
Exercise
1st
Exercise
r • 0·97
P < 0·05
Before Exercise
0
10
20
30
40
PLASMA IRI (μ units/ml)

rapidly to a peak at the end of the first period of exercise and then fell in the recovery period. The concentrations rose again during the second period of exercise but to a much greater extent, and remained elevated both during the remaining part of exercise and for 15 min afterwards. The concentrations then fell progressively to pre-exercise values 60 min later. In the control group ketone-body concentrations changed little during the two periods of exercise, but they rose after the end of each period, more so after the second.

Plasma IRI (Fig. 5.2): Resting concentrations of plasma IRI were higher in the patients than in controls. The concentrations fell in the control subjects during the two periods of exercise and rose slightly above their initial values in the second post-exercise period. The patients however showed a rapid rise in the concentrations of IRI both at the end of the first exercise period and the end of the second period. The rise was much greater on the second occasion. In the acromegalics plasma IRI concentrations returned to resting values 60 min after each period of exercise.

DISCUSSION

There were considerable differences in the pattern of ketone-bodies and insulin changes during exercise between the two groups. The patients had a considerable rise in blood ketone-bodies which was absent in the control subjects. Plasma IRI concentrations were increased in the patients, whereas in controls they were depressed. These changes were remarkable and were similar to the observations of Johnson and Rennie (1973). Throughout exercise for 30 min by acromegalics Johnson

and Rennie found no change in blood glucose concentrations and therefore the changes in plasma IRI are not related to hyperglycemia.

Growth hormone reduces tissue sensitivity to insulin and the higher than normal concentrations of plasma HGH which occur in acromegaly may indirectly result in greater amounts of insulin being released. However a rise in plasma IRI was absent in the controls despite a greater elevation of HGH in their blood during the second period of exercise. Other factors must therefore be involved in causing insulin release. The increased IRI concentration in the acromegalic patients might be in response to the rapid increases in ketone-bodies. These are known to stimulate insulin release and observations are described in chapter 4. Evidence is provided for this explanation by the high correlation ($r. 97$; $p < 0.05$) between the highest observed concentrations of plasma IRI and blood ketone-bodies in the acromegalics (Fig 5.3). After exercise the blood ketone-bodies fell in the patients so that no post-exercise ketosis developed. This may have been related to the rise in plasma IRI as insulin is known to limit fat mobilization and therefore inhibits ketone body production (Bieberdorf et al., 1970). The present observations imply that the action of ketone-bodies in stimulating insulin release and the ability of insulin to suppress ketone-body production are active in spite of high concentrations of HGH. These findings support conclusions based on animal work (Jenkins, 1967) that the explanation for the raised plasma insulin concentrations associated with reduced uptake of glucose often seen in certain diseases such as in obesity and diabetes may depend upon the high plasma FFA and blood ketone-body concentrations found in these disorders.

SUMMARY

1. Observations upon the relationship between ketone-bodies and insulin release at rest have been reported in chapter 4 and this chapter reports studies on ketone-bodies and plasma insulin during and after two periods of exercise in two patients with acromegaly. Three normal subjects were also studied as controls.

2. In the acromegalics ketone-bodies concentrations rose during exercise and fell in the post-exercise period, whereas in the controls the concentrations changed little during exercise but rose above resting values immediately after exercise. The rises in ketone-bodies in both groups were associated with proportionate rises in plasma IRI concentrations. These changes in blood ketone-bodies and plasma IRI were more marked during and after the second period of exercise in both groups.

3. These observations support the previous finding (chapter 4) that ketones stimulate insulin release in man. They also provide more evidence for the suggestion that ketones prevent ketosis by feed-back control dependent on insulin release.

CHAPTER 6

PATIENT WITH MUSCLE DISEASE

(Mitochondrial inclusion body myopathy)

INTRODUCTION

Experience gained in carrying out the projects reported in the previous chapters on muscle metabolism during exercise in normal subjects and in patients with obesity have made it possible to examine fat and carbohydrate metabolism in patients with diseases of muscle. One disorder which has been studied is that of oculoskeletal myopathy, patients with which, present with progressive ptosis and external ophthalmoplegia. In this disorder electron microscopy has allowed delineation of intracellular abnormalities affecting organelles. A number of studies have reported abnormal histochemical and ultrastructural changes inside the mitochondria of muscle cells affected (Bradley et al., 1969; Morgan-Hughes and Mair, 1973). There is, however, no information about the effect of the disorder upon metabolic processes. Investigations were designed, therefore, to provide more information about energy metabolism in this disease.

This work was carried out in conjunction with Dr. D. Doyle (Neuropathologist, Southern General Hospital) who has performed the biopsies and is responsible for histological and histochemical studies, but I am only responsible for the biochemical studies both at rest and during exercise.

CASE REPORT

AB., (Institute of Neurological Sciences, Glasgow, 807875) was a 49 yr old married man with two children, who was employed as a lorry driver. His mother died of carcinoma of the stomach at 62 yr, and his father of a myocardial infarction at 67 yr. He had five siblings, three of whom were well, one had died of alcoholic cirrhosis (50 yr) and the other of 'stomach trouble' (51 yr). There was no family history of muscle disease, ptosis, or diabetes. He complained of drooping of his eyelids for many years, and he was aware of trying to lift them with his forehead. He had also had very occasional double vision on lateral gaze for about 2-3 months. These symptoms were worse later in the day. Photographs indicated that he looked normal at 18 yr, but a left ptosis had become obvious at about 33 yr. He made no complaint about tiredness when first seen, but later admitted that this had become a problem for 1-2 yr; it was not related to time of day.

On examination his height was 176 cm and weight 62 kg. There was no obvious muscle wasting. He had wrinkling of his forehead, bilateral ptosis, and there was weakness of upward conjugate gaze and slight weakness of conjugate gaze to either side. Convergence was poor. His voice had a nasal quality but the palate moved normally. In the limbs there was no muscle fatiguability but slight weakness of both triceps muscles and of the small muscles of both hands. All reflexes were normal. The remainder of the physical examination was negative.

Muscle Biopsies

Biopsies were taken from the left triceps and the left deltoid

muscle under local anaesthetic. They were examined by light microscopy with routine stains. Glycogen distribution was identified by periodic acid Schiff staining (Hotchkiss, 1948) and fat by staining with Oil red O (Lillie and Ashburn, 1943). Specimens were also examined by electron microscopy (Philips 201 electron microscope), goniometry (Philips 301 electron microscope with double tilt goniometry), electron diffraction and X-ray microchemical analysis ('Edax' (Energy Dispersive Analysis by X-ray)) with Philips 301 electron microscope. Histochemical analysis was carried out for the enzymes shown in table 6.1. Succinic dehydrogenase was examined by the method of Seligman and Rutenburg (1951); myosin ATPase by that of Padykula and Hermann (1955a, b); glucosan phosphorylase by that of Takeuchi and Kuriaki (1955); and leucine aminopeptidase by that of Nachlas et al., (1957). The other enzymes were studied by the methods of Scarpelli et al., (1958).

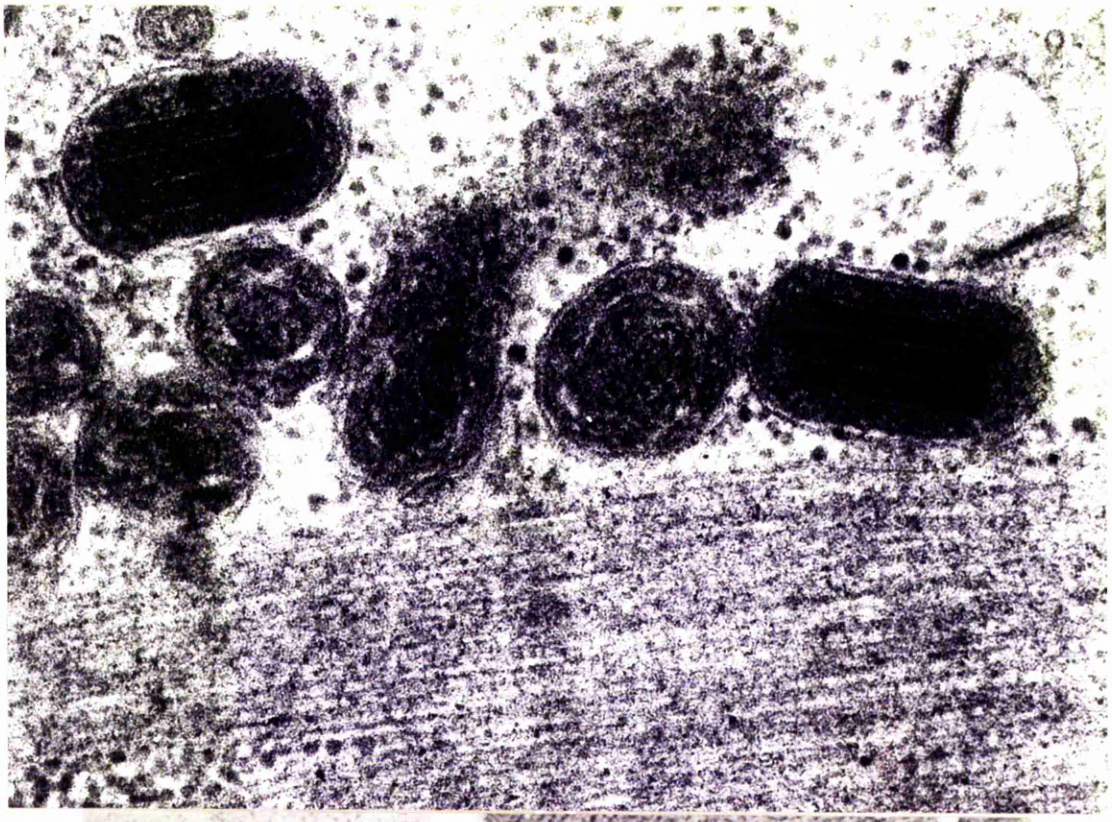
Light microscopy with phase contrast and polarised light revealed virtually normal muscle and the abnormalities seen in stained preparations were minor. In the specimen from the left triceps muscle about 5% of the fibres were atrophic, some being exceptionally small in cross sectional area (20 μ) with clusters of peripheral nuclei. In atrophic fibres there were also some nuclei which were larger than normal (15 μ diameter) and these had very large nucleoli (5 μ diameter). Some obviously abnormal fibres (approximately 2% of total) had unusually pale borders which had abnormal mitochondrial aggregates. The nuclei and nucleoli were larger in these areas. The remainder of the muscle fibres, although appearing normal, occasionally had internally placed nuclei and in a few scattered areas these occurred in 50% of the fibres. Longitudinal

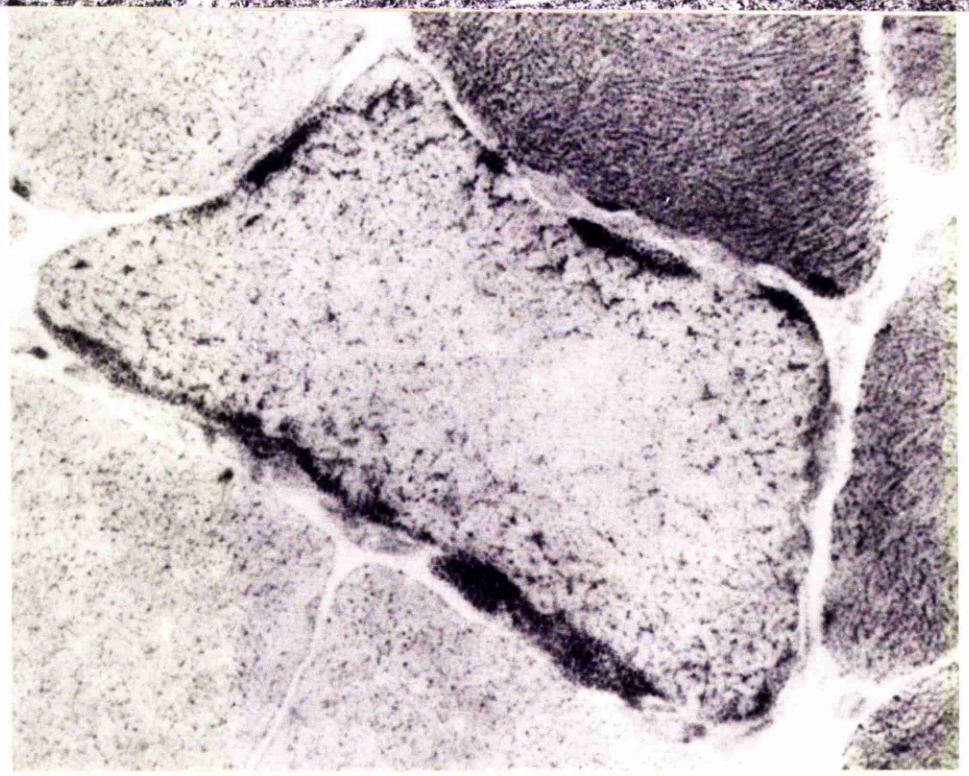
section showed that some nuclei were concentrated close to capillaries and these nuclei appeared larger than normal. The changes in the specimen from the left deltoid muscle were similar but only 1% of the fibres were atrophic. Atrophic fibres appeared in groups of up to six but no evidence was found of denervation as motor end plates and terminal innervation appeared normal. There was a larger proportion of fibres with pale borders in this specimen than in that from triceps (about 5%). In longitudinal section large nuclei were frequently seen in relation to capillaries. In both specimens glycogen was found to be reduced in all fibres. The concentration of fat in type I fibres was reduced and it was virtually absent from type II fibres: the fat which remained was mainly peripheral in the fibres.

Electron microscopy (EM) (fig 6.1) of both specimens revealed no normal mitochondria. The least abnormality was the arrangement of cristae in concentric circles in the mitochondria rather than transversely as is found normally. Some mitochondria also showed crystal-like inclusions. These mitochondria were most numerous in the pale peripheral borders of the abnormal fibres seen on light microscopy. The crystals appeared to be invested by the inner mitochondrial membrane which was not incorporated in their structure. The crystals varied in length from 0.2 to 5 μ and were slightly curved. They were sometimes square but were usually oblong. Each crystal consisted of bands about 0.04 μ in width separated from adjacent bands by a clear region 0.008 μ across. The bands had light and dark longitudinal stripes (0.008 μ side). Although the majority of crystals had such a pattern, in some the bands were arranged obliquely and others had a grid-like structure. Goniometry confirmed that some of the crystals were curved and it was possible to

Fig (6.1): Electron micrograph of the crystalline inclusion bodies in mitochondria showing the patterns which are determined by the angle of incidence of the electron beam. Two mitochondria are shown which do not contain crystals but have abnormally arranged cristae (X 12,500).

Fig (6.2): Abnormal succinic dehydrogenase activity in a type I muscle fibre. The intense zone of peripheral activity was characteristic of the abnormal fibres. (X 700).





show that the differences in the appearances of the bands depended upon the angle of incidence of the electron beam on the crystal. Electron diffraction and microchemical X-ray analysis were non-contributary because of the low mass of the crystals. EM confirmed that there was a general reduction in the fat content of the muscle fibres.

Histochemical examination showed the usual distribution of fibre types with myosin ATPase, fibres of types I, II and IIa being distinguished. Fibre typing however was more difficult with other enzymes. Marked abnormalities were observed in fibres of types I and IIa, 20% of all fibres being affected, but fibres of type IIb were not clearly seen. No abnormalities were found in type II fibres. Abnormalities were principally seen in the activities of mitochondrial or predominantly mitochondrial enzymes but there were also changes in several enzymes in cytoplasm. In the biopsy from triceps there was a general reduction in the activity of succinic dehydrogenase but there was a peripheral zone of considerably increased activity in type I and type IIa fibres. In the biopsy from deltoid the activity of succinic dehydrogenase was also increased peripherally in type I and type IIa fibres (fig 6.2). Several other mitochondrial enzymes showed increased activity and similar changes were observed in cytoplasmic enzymes, including lactate dehydrogenase.

Biochemical Response to Exercise

The biochemical changes during exercise in our patient were studied on two occasions during and after a 30 minute period of exercise. The results were compared with those from 6 normal male subjects (aged 28-44 yr) as controls. The investigations were performed between 09.00 hr

Table 6.1:

Enzyme	Site of Enzyme	Activity in Muscle Fibres				Distribution in Abnormal Fibres according to type		
		Cytoplasm		Mitochondria				
		normal fibres	abnormal fibres	normal fibres	abnormal fibres	I	II	IIa
succinic dehydrogenase	M	t* – d –	– –	reduced N	++ +++	++ +++	N "	++ ++
cytochrome oxidase	M	–	–	"	+++	+++	"	++
B hydroxy butyrate	M	–	–	"	+++	+++	"	++
glutamate dehydrogenase	C + M	N	N	"	+++	+++	"	++
isocitrate dehydrogenase	C + M	"	"	"	++	++	"	+
α glycerophosphate dehydrogenase – NAD linked	C + M	"	"	"	++	++	"	+
NAD – tetrazolium oxido-reductase	C + M	"	"	"	+++	+++	"	++
NADP – tetrazolium oxido-reductase	C + M	"	"	"	+++	+++	"	++
malate dehydrogenase	C + M	"	+++	"	+++	+++	"	++
lactate dehydrogenase	C	"	+++	–	–	+++	"	++
glucose-6-phosphate dehydrogenase	C	"	+++	–	–	+++	"	++
6-phosphogluconate dehydrogenase	C	"	+++	–	–	+++	"	N
alcohol dehydrogenase	C	"	+++	–	–	+++	"	+
myosin ATPase	C	"	N	–	–	N	"	N
glucosan phosphorylase	C	"	"	–	–	"	"	"
leucine aminopeptidase	C + M	"	"	N	N	"	"	"

and 11.00 hr after an overnight fast. A catheter was placed in an antecubital vein and flushed with saline. Two resting samples were taken. Work was performed on a bicycle ergometer (Elma Schönander Constant load ergometer, EM369) at 600 kpm for 30 min. Heart rate was recorded during the investigation and for a further 15 min, using miniature chest electrodes with an electrocardiograph. Blood samples were taken at 5 min intervals during exercise and then at 15, 30, 60 and 90 min afterwards. The controls were investigated in a similar way, but their work load was adjusted to obtain a comparable increase in heart rate to that in the patient (500 $\pm$ 100 kpm). In the second investigation expired air was collected in a Douglas bag and analysed for O_2 and CO_2 to determine oxygen consumption and respiratory quotient (R). Measurements were made at rest and during exercise and recovery. The blood samples were analysed as described in appendices I and II.

Similar maximum heart rates were achieved during exercise.

Glucose (fig 6.3). Concentrations at rest were similar in the patient and the controls but there was a marked difference during and after exercise. The blood glucose rose to 135 mg% in the patient at the end of exercise, whereas there was no significant change in the controls.

Lactate (fig 6.4). Concentrations at rest were similar but with exercise there was a rapid elevation in the patient to 12.5 μ mol/ml compared with 4.0 μ mol/ml in the controls. The concentration in the patient declined after exercise but had not returned to the resting value 90 min after the end of exercise.

Pyruvate (fig 6.4). Concentrations at rest were similar and the exercise

Fig (6.3): Plasma IRI (μU/ml) and blood glucose (mg% in the patient (●——●) and six normal control subjects (0-----0) (± SEM) during and after 30 min of exercise, indicated by the solid bar.

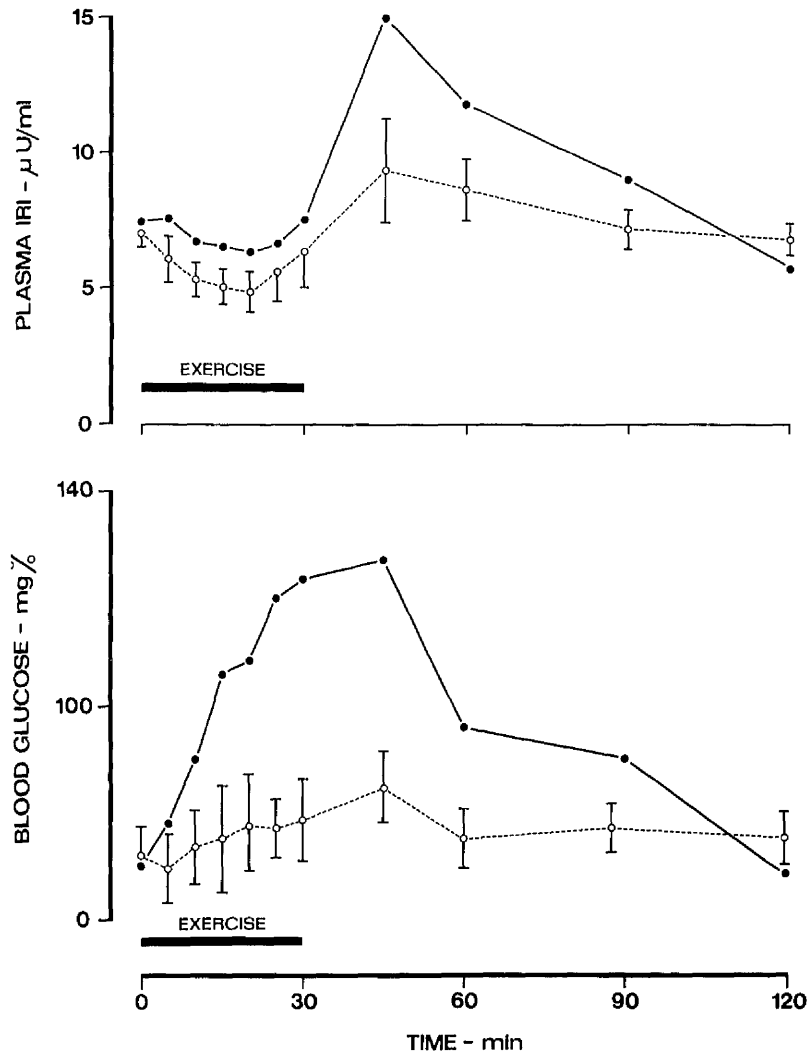
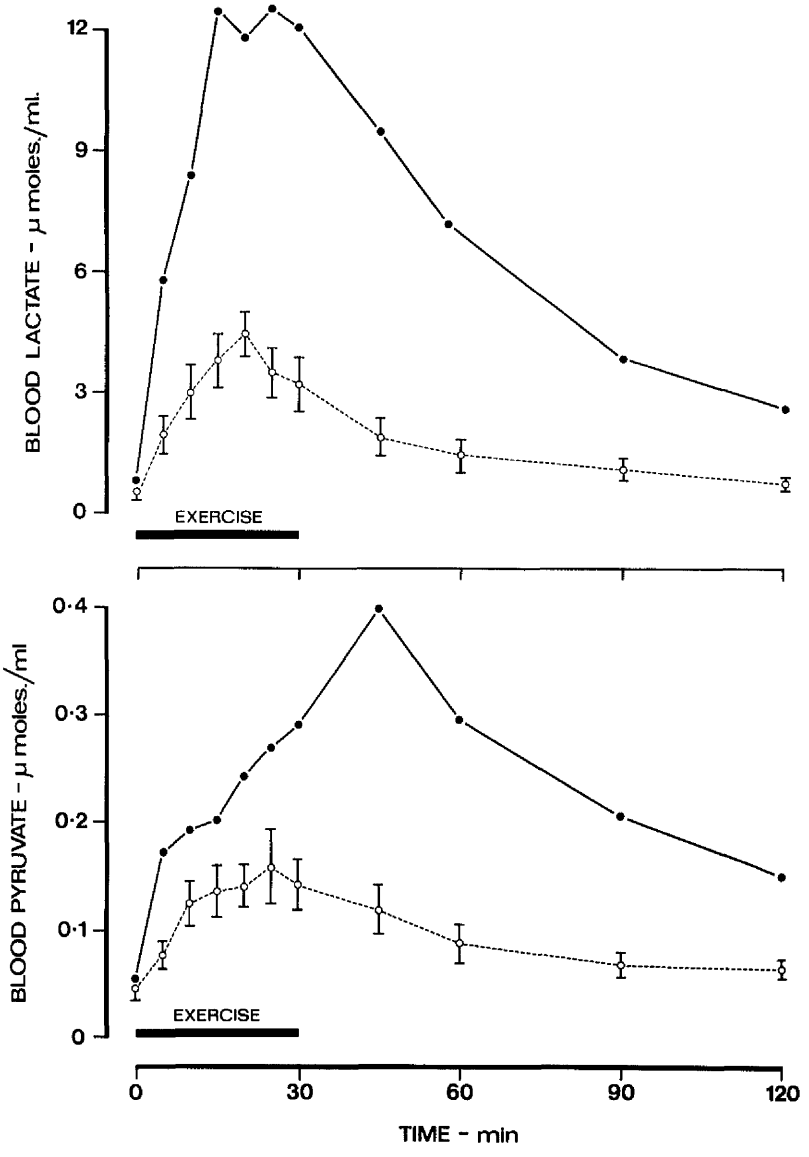

15
10
5
0
PLASMA IRI - μU/ml
EXERCISE
140
100
0
BLOOD GLUCOSE - mg%
EXERCISE
0
30
60
90
120
TIME - min

Fig (6.4): Blood lactate and pyruvate (μ mol/ml) in the patient (⊗———●) and six normal control subjects (0-----0) (± SEM) during and after 30 min of exercise, indicated by the solid bar.


BLOOD LACTATE - μ moles./ml.
12
9
6
3
0
EXERCISE
BLOOD PYRUVATE - μ moles./ml
0·4
0·3
0·2
0·1
0
EXERCISE
0
30
60
90
120
TIME - min

caused a rapid elevation. The highest concentration in the patient (0.39 μ mol/ml) was achieved 15 min after the end of exercise. After this the concentration declined but had not returned to the resting value by the end of the investigation. The maximum lactate/pyruvate ratios were greatly elevated in the patient (400% above resting) compared with the controls (230% above resting).

Plasma FFA (fig 6.5). Concentrations at rest in the patient were 80% higher than in the controls on the first occasion but similar on the second. On both occasions concentrations declined during exercise compared with the controls, in which they rose.

Blood glycerol (fig 6.5). Concentrations at rest in the patient were higher than in the controls on the first occasion but similar on the second. In both studies they rose to nearly twice the mean level in the controls by the end of exercise. The concentration then declined in both the patient and the controls, the resting values being achieved by the end of the investigations.

Total blood ketone-body (fig 6.6). Concentrations at rest in the patient were five times higher than in the controls on the first occasion and within the normal range on the second. The concentrations fell during and after exercise whereas they rose in the controls. On the first occasion the 3-hydroxybutyrate/acetoacetate ratio for the patient was 2.24 before exercise and rose to 4.40 after 5 min of exercise and then fell progressively to 1.0 30 min after exercise. In the second exercise investigation on the patient the changes were similar. In the controls the mean value at rest was 2.16 and it fell to 1.36 after 5 min of exercise. After 30 min of exercise it was 2.83 and 30 min later it had risen to 3.8.

Fig (6.5): Plasma FFA (μ equiv/ml) and blood glycerol (μmol/ml) in the patient (●———●) and six normal control subjects (0------0) (± SEM) during and after 30 min of exercise, indicated by the solid bar.

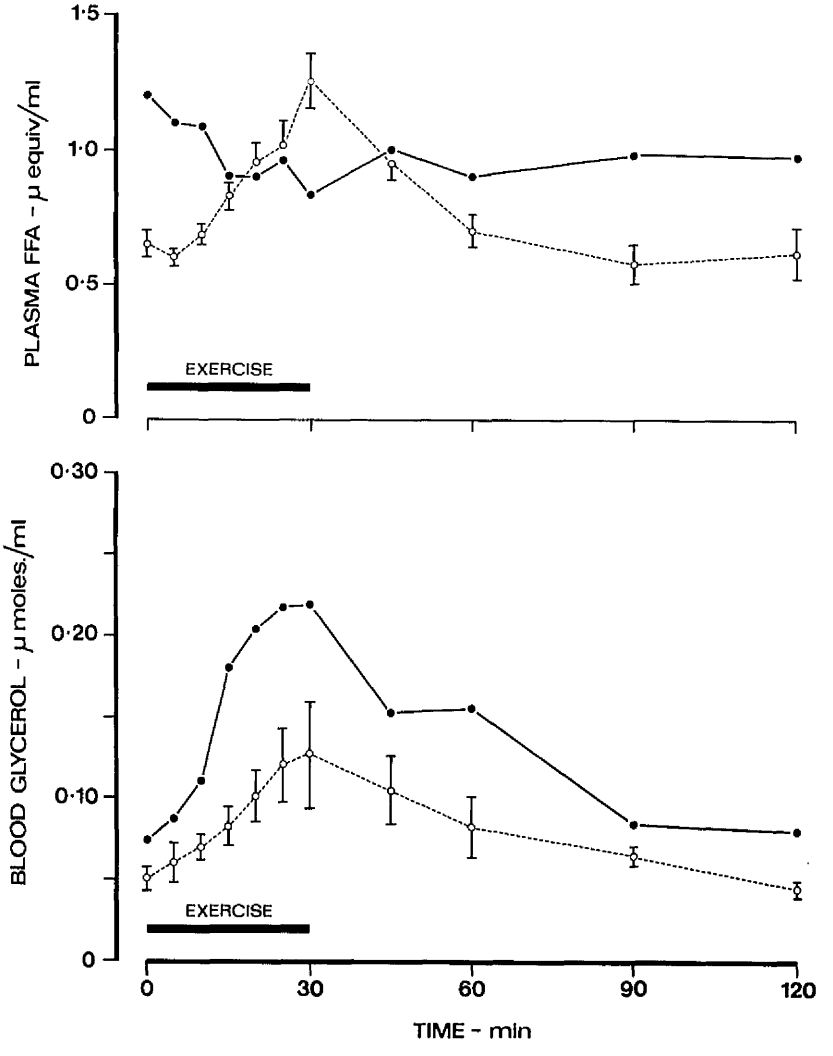
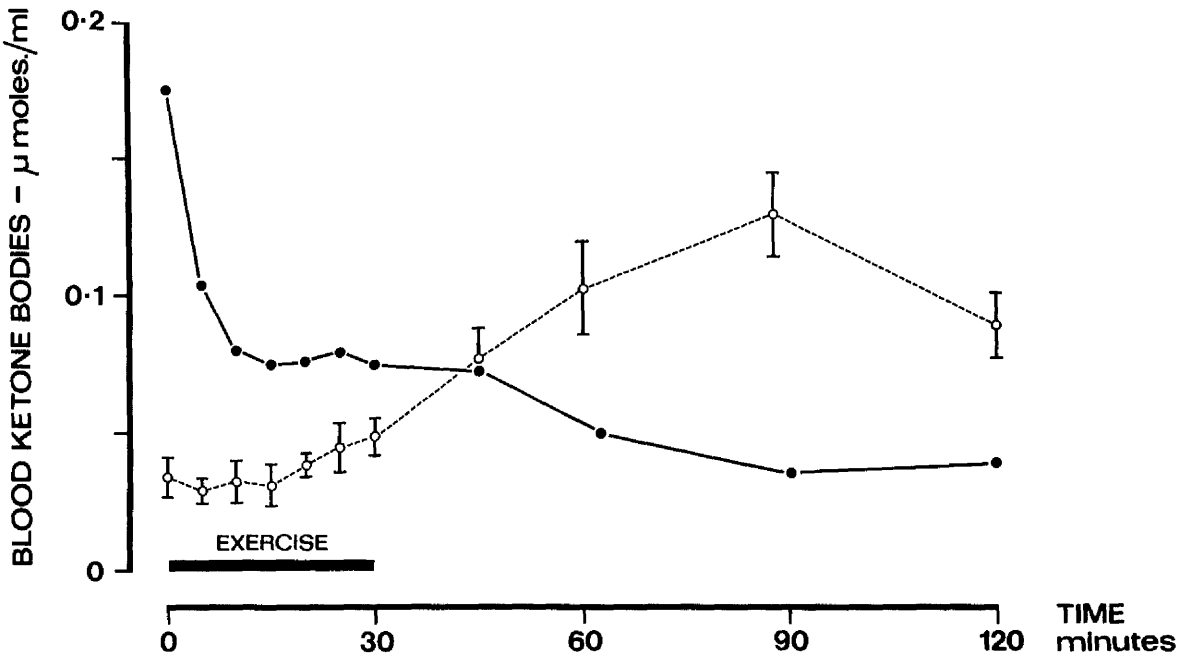

1·5
1·0
0·5
0
PLASMA FFA - μ equiv/ml
EXERCISE
0·30
0·20
0·10
0
BLOOD GLYCEROL - μ moles./ml
EXERCISE
0
30
60
90
120
TIME - min

Fig (6.6): Blood ketone-bodies (3-hydroxybutyrate + acetoacetate; μ mol/ml) in the patient (●———●) and six normal control subjects (O-----O) (± SEM) during and after 30 min of exercise, indicated by the solid bar.


BLOOD KETONE BODIES – μ moles/ml
0·2
0·1
0
EXERCISE
0
30
60
90
120
TIME
minutes

Plasma IRI (fig 6.3). Concentrations at rest were similar but the exercise caused a fall in plasma IRI in all studies. The fall was greater in the controls after 20 min of exercise. After this the concentrations rose, both in the remaining part of exercise and for 15 min afterwards. The concentration was much greater 15 min after exercise in the patient (200% of resting) than in the controls (133% of resting). The concentrations returned to resting values by the end of the investigations.

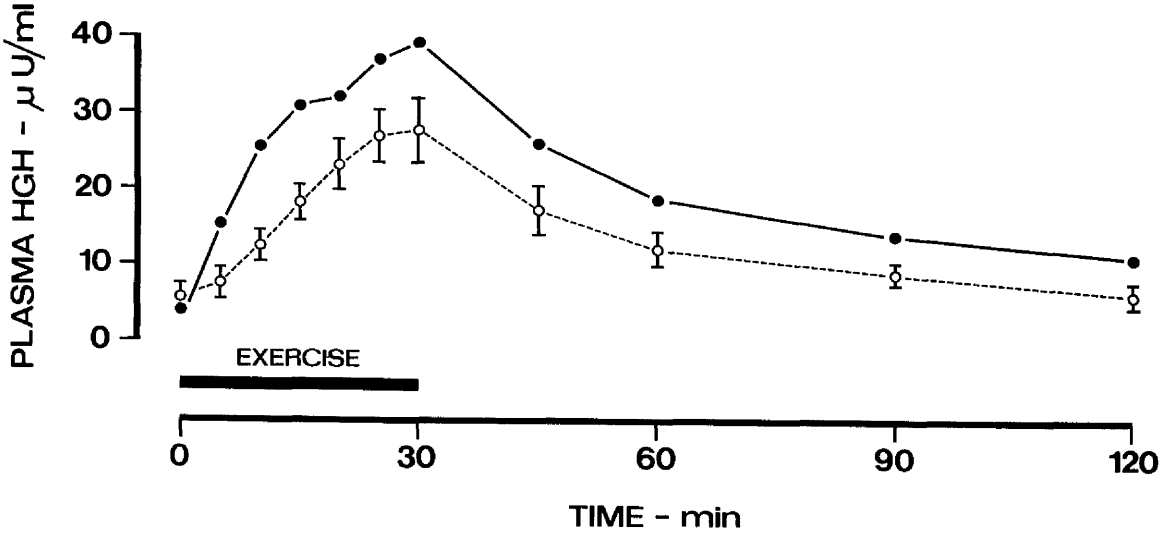
Plasma HGH (fig 6.7). Concentrations at rest were similar but with exercise there was a rapid elevation in the patient to 40 μU/ml compared with 27 in the controls. The concentrations then declined but had not returned to the resting values by the end of the investigations.

Oxygen consumption at rest was 0.24 l/min. The values at 6, 20 and 30 min during exercise were 1.54 l/min, 1.38 l/min and 1.45 l/min. The oxygen consumption then returned to normal 20 min after the end of exercise. R values were 1.04 at rest, they fell progressively during exercise to a minimum of 0.74 at the end of exercise. The value 20 min afterwards was 0.84.

The effect of heparin upon lipolysis

The investigations were carried out in the morning after an overnight fast according to the method of Fineburg et al., (1972). Corn oil (60 g) which had been emulsified with egg albumin (15 g) was given orally. This was followed 3 hours later by an intravenous injection of heparin (50 mg). Venous blood samples were taken before ingestion of the corn oil, at 30 min intervals and then at 15 min intervals after the injection of heparin for a further 2 hours. The test was repeated on

Fig (6.7): Plasma HGH (μU/ml.) in the patient (●———●) and six normal control subjects (0------0) (± SEM) during and after 30 min of exercise, indicated by the solid bar.


PLASMA HGH - μU/ml
40
30
20
10
0
EXERCISE
0
30
60
90
120
TIME - min

another occasion omitting the corn oil. The blood samples were analysed for FFA and ketone-bodies (acetoacetate plus 3-hydroxybutyrate) as already described. Following the ingestion of the corn oil plasma FFA concentrations remained unchanged and just prior to the injection of heparin the concentration was 0.80 μequiv/ml. The concentration 45 min after the injection of heparin was 2.4 μequiv/ml and it then fell to a value of 1.8 μequiv/ml 25 min later. In the control investigation on the patient injection of heparin alone also caused a rise in the concentrations of FFA, but this was less than that obtained after ingestion of corn oil. The rise in FFA concentrations after heparin were associated with a considerable rise of total ketone bodies.

Glucose tolerance test

A standard glucose tolerance test was performed after an overnight fast. Glucose (50 g) was given orally and blood samples were obtained at 30 min intervals for 2½ hours. Lactate, pyruvate, glucose and plasma IRI were estimated as in the exercise investigation. The rise and the subsequent fall of both glucose and plasma IRI were similar to those of normal subjects. The tolerance to a glucose load was therefore normal in the patient.

DISCUSSION

Abnormal muscle mitochondria have been reported in several apparently unrelated conditions. The first report of a muscle disorder with abnormal mitochondria was made in 1959 when increased metabolism was found. The patient's mitochondria contained large crystalline inclusions (Ernster et al., 1959; Luft et al., 1962). Mitochondrial

abnormalities without associated hypermetabolism have been reported in childhood (Shy et al., 1966). In one of these, 'megaconial myopathy', rectangular inclusion bodies were observed in the muscle mitochondria. Mitochondrial inclusions have also been reported in the skeletal muscle of adults (Shafiq et al., 1967; Bradley et al.,1969). None of these reports included observations upon the effect of the muscle disorder upon metabolism.

In this study the patient had an abnormal metabolic response to exercise compared with controls. Concentrations of lactate and pyruvate during exercise rose to values above those observed in the controls. He also showed a rapid rise in blood glucose concentration. These observations suggest that although glycolysis was proceeding there was a block to complete oxidation of carbohydrate via acetyl CoA and the tricarboxylic acid cycle.

The lactate/pyruvate ratio rose during the first part of exercise in the patient above those observed in the controls. Some elevation is the usual response and is probably due to a rapid fall in the ratio of cytoplasmic nicotinamide adenine dinucleotide/reduced nicotinamide adenine dinucleotide (NAD/NADH) (Krebs, 1967). This cytoplasmic activity is normally linked to the concentration of NAD/NADH in mitochondria but may be independent (Devlin and Bedell, 1960). These changes in the mitochondria may be reflected in the ratio of the concentrations of 3-hydroxybutyrate/acetoacetate in the blood (Williamson et al., 1967). In the patient this ratio first rose and then fell,

whereas the converse was observed in the controls. Such findings are obtained with relative cellular hypoxia (Alberti et al., 1971). The changes in these ratios are therefore in keeping with the suggestion already made of a block of oxidative metabolism for carbohydrate.

There was also a much greater rise in blood glycerol concentration in the patient compared with controls suggesting greater fat mobilization in the patient. Lipolysis results in production not only of glycerol but also FFA. The rise in the concentration of plasma FFA during exercise which occurred in the controls, and is the normal finding, was, however, absent in the patient. Tests of lipoprotein lipase activity had shown that there was no block in the normal production of FFA. The depression of plasma FFA in the patient during exercise may therefore have been due to either greater catabolism of FFA or re-esterification to fat. There was no evidence from the muscle biopsies that deposition of fat was occurring excessively in the muscles studied. Normally ketone-body concentrations rise during the post-exercise period (Courtice and Douglas, 1936; Johnson et al., 1969) but this change was absent in the patient. The block of carbohydrate metabolism could result from increased fat oxidation to acetyl CoA, a process which is known to block further pyruvate oxidation (Garland and Randle, 1964) and cause accumulation of pyruvate and lactate. This would also account for some of the elevation of glycerol for this is normally metabolised through the glycolytic pathway via α-glycero-phosphate.

The rapid rise of blood glucose implies both decreased carbohydrate utilization during exercise and also active glucose production by the liver. Pathways subserving carbohydrate storage as glycogen were

normal, however, as adduced from the normal glucose tolerance test. The block of carbohydrate metabolism did not prevent the patient exercising and it is probable that energy production depended upon metabolism of fat as mobilization was occurring and yet there was no accumulation of FFA during exercise and of ketone-bodies after exercise. Additional support for the conclusion that fat was actively metabolised by the patient was the finding that the patient had a relatively low R (CO_2 production/O_2 consumption) during exercise. R normally rises in severe exercise to a value of 1.5 - 2.0. The value above unity before the investigation might result from hyperventilation but it would be unlikely that this would have driven off so much CO_2 prior to exercise that 30 min later the R would still be below normal values, as occurred. This argument is also supported by the histochemical findings of increased activities of enzymes involved in oxidative metabolism such as succinic dehydrogenase and cytochrome oxidase inside the abnormal mitochondria.

There were also differences in the hormonal responses to exercise of the patient compared with the controls. Plasma IRI concentration was depressed to a lesser extent in the patient during exercise compared with the controls and he had a greater increase after exercise. During exercise there is an increase in circulating catecholamines (von Euler and Hellner, 1952), which may explain the failure of the increased glucose concentration in the patient to stimulate insulin release, as adrenaline and nor-adrenaline inhibit insulin release (Kris et al., 1966; Porte and Williams, 1966). The higher concentrations of plasma IRI in the patient compared with controls after exercise could,

however, be related to the increased blood glucose as concentrations of blood catecholamines return rapidly to normal, the fall being of the order of 60% in 5 min (Johnson et al., 1974).

Plasma HGH concentrations were higher in the patient during exercise. The role of HGH during exercise has not been clearly established. It has been suggested that it causes lipolysis and enhances the availability of FFA (Hunter et al., 1965) and therefore the difference in FFA response to exercise in our patient compared with the controls may be related to the differences in HGH concentration. However studies of patients with hypopituitarism have shown that it is not essential for FFA release (Johnson et al., 1971).

There appear to be considerable differences in the metabolic effects of disorders in which abnormal mitochondria are found. The patient with myopathy described by Bradley et al., (1969) had mitochondria changes on EM which appeared similar to those in the patient reported in this study. However in contrast, they found excessive fat in muscle fibres. The two patients described by Coleman et al., (1967) had increased activity of succinic dehydrogenase and other enzymes but no EM studies were reported. These patients however also had abnormal accumulation of fat in muscle fibres. Mitochondrial aggregates have been described by Engel (1964) in the muscle fibres from three patients, but these mitochondrial aggregates lacked succinic dehydrogenase activity, thus differing from the abnormal mitochondria in the patient reported in this study. The patient described by Luft et al., (1962) had increased metabolic activity and they considered that this occurred in spite of a defect in mitochondrial enzyme organisation. The increased fat metabolism

during exercise which we now report is compatable with their findings. These observations indicate that exercise provides a useful tool for examining the metabolism of abnormal mitochondria. An abnormal metabolic response to exercise has been found and I suggest that studies of further patients may allow delineation of specific patterns of metabolism of value in diagnosis.

SUMMARY

1. A male patient (49 yr) presented with bilateral ptosis of 15-20 yr duration and weakness for 1-2 yr. Muscle biopsies were performed from the deltoid and triceps muscles and studied by means of histology, histochemistry and electron microscopy.

2. Routine histology showed only minor changes; 2-5% of muscle fibres had pale borders in which there were aggregates of mitochondria and 1-5% of fibres showed atrophy. Histochemical examination showed increased activity of succinic dehydrogenase in mitochondria and lactate dehydrogenase in cytoplasm. Electron microscopy showed crystalline inclusions in many subsarcolemmal mitochondria.

3. Metabolites were studied during and after exercise on an ergometer and revealed remarkable differences from normal. Blood lactate rose to 12.5 μ mol/ml and pyruvate to 0.39 μ mol/ml compared with up to 4.0 and 0.16 μ mol/ml respectively in controls. Concentrations of ketone-bodies and free fatty acids fell during and after exercise whilst they rose in the controls.

4. These observations imply a major mitochondrial defect which causes dramatic biochemical changes in fuel supply in blood during exercise. The changes suggest that fat metabolism was accelerated in this patient and was related to a block of carbohydrate utilization as fuel.

CHAPTER 7

A COMPARISON OF TREATMENT OF OBESITY WITH FENFLURAMINE AND WITH DIETARY RESTRICTION

INTRODUCTION

The first part of this thesis has provided evidence that obesity causes marked changes in the patterns of carbohydrate and fat metabolism during exercise. Compared to healthy subjects, fat people show higher concentrations of blood glucose and glycerol during exercise and blood ketone bodies during and after exercise (original observations together with discussion are given in chapter 2). As there are differences in blood concentrations of metabolites the pattern of utilization of fuel by working muscle is probably altered as a result of obesity. In obesity there seems to be a block to muscle glucose uptake which is related to the degree of fatness (Butterfield et al., 1965). In this situation if muscle is not used for glucose disposal, the glucose must presumably be stored as fat in adipose tissue for use when required by muscle. When dietary measures alone fail to correct the metabolism in obesity, drugs such as amphetamines have been used, but their value is short lived and amphetamines have the side effect of stimulating the nervous system. Recently fenfluramine, a derivative of amphetamine but said to have fewer side effects was introduced as an appetite depressant in the treatment of obesity (Munro et al., 1966). Several investigations have suggested, however, that part of its action is related to a direct effect on carbohydrate and fat metabolism. The drug improves glucose tolerance, possibly because it increases glucose uptake by muscle (Butterfield and Whichelow, 1968). There is also evidence for a direct effect on adipose tissue. Fenfluramine has a lipolytic activity and may depress lipid synthesis in vitro (Dannenburg and Kardian, 1967; Wilson and Galton, 1971). During therapy with the drug there are increases in

concentrations of FFA, glycerol and total ketone-bodies (Pawan, 1969). Whether these effects of fenfluramine are direct or secondary to dietary restriction has been unclear. The present study was undertaken to examine further the effect of fenfluramine on fat and carbohydrate metabolism in man and to compare the results with the effect of dietary restriction, which has been shown in chapter 3 to also cause marked increase in fat metabolites in blood.

METHODS

Subjects

A. Treated with fenfluramine: (Table 7. 1A) Six volunteer subjects who were overweight (5 male, 1 female, aged 24-47 yr) were studied for five weeks while taking fenfluramine. They took an unrestricted diet and carried out their usual activities. The dose of fenfluramine was 40 mg in the first week, 60 mg for the second and third weeks and 80 mg for the last two weeks.

B. Treated with partial or complete starvation: (Table 7. 1B) Four subjects (2 male, 2 female, aged 22-60 yr) undergoing dietary restriction therapy for obesity were also studied. Two of the patients received a diet of 600 calories for 12 days and two received water only with added vitamins, for five weeks.

C. Treated with fenfluramine and reduced diet: (Table 7. 1C) Two subjects who were overweight were studied. One of these (C. McH.) was given a 600 calorie diet for 24 days. In the second half of the period he also received fenfluramine (100 mg daily). The second subject

	Sex	Age (Yr)	Height (cm)	Weight (kg)	Weight Loss (kg)	Reciprocal Ponderal Index ($ht/\sqrt[3]{wt}$)	% increase above ideal wt.
A. SUBJECTS TREATED WITH FENFLURAMINE							
W.S.	M	31	168	80.6	6.1	39.63	30
M.R.	M	24	188	94.6	7.7	41.46	23
J.B.	M	30	180	88.0	4.9	40.44	28
R.J.	M	36	173	73.5	2.6	41.62	24
J.W.	M	26	182	127.0	6.0	36.18	76
M.McE.	F	47	158	91.0	4.0	35.07	82
B. SUBJECTS TREATED WITH DIETARY RESTRICTION							
E.H.	F	60	163	110.5	18.5	33.95	78
H.W.	M	22	170	125.0	20.00	34.00	84
J.D.	F	34	165	78.6	4.3	39.00	30
C.McH.	M	51	180	92.3	5.2	39.82	27
C. SUBJECTS TREATED WITH A COMBINATION OF DIETARY RESTRICTION AND FENFLURAMINE							
S.T.	M	56	170	96.0	5.3	37.03	46
C.McH.	M	51	180	92.3	5.2	39.82	27

Table 7 : 1 Individual data on age, height, weight and reciprocal ponderal index

(S.T.) was also given 600 calories for 24 days but received fenfluramine (100 mg) daily for the first 12 days.

Procedure:

Body weight was measured at intervals for five weeks. Venous blood was taken between 09.00 and 09.30 hr after an overnight fast, at the beginning and at four day intervals throughout the investigation period. Blood samples were treated as described in appendix I and analysed for glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glycerol and free fatty acids.

RESULTS

All subjects were overweight, but the group who underwent dietary restriction were somewhat shorter and heavier than those receiving fenfluramine, as judged from their reciprocal ponderal indices ($ht/\sqrt[3]{wt}$) or their % wt above their ideal wt (Metropolitan Life Insurance Co. figures quoted in Giegy, 1962).

Subjects treated with fenfluramine compared with those treated with dietary restriction

With both treatments weight loss occurred. Those undergoing dietary restriction lost more (4.3 - 20.0 kg) than those receiving fenfluramine (2.0 - 7.7 kg : table 7.1). During the five weeks of investigation the concentrations of plasma FFA (Fig 7.1) increased considerably. The concentrations were much higher in those undergoing dietary restriction ($p < 0.01$). The changes in blood glycerol concentrations (Fig 7.1) were similar to the changes in plasma FFA.

Fig (7.1): Plasma FFA (μ equiv/ml, mean ± SEM), blood glycerol and total ketone-bodies (μ mol/ml, mean ± SEM), during fenfluramine therapy (6 obese subjects, ■) and dietary restriction (4 obese subjects, ●), as only two subjects underwent dietary restriction for more than 12 days their individual results are shown.

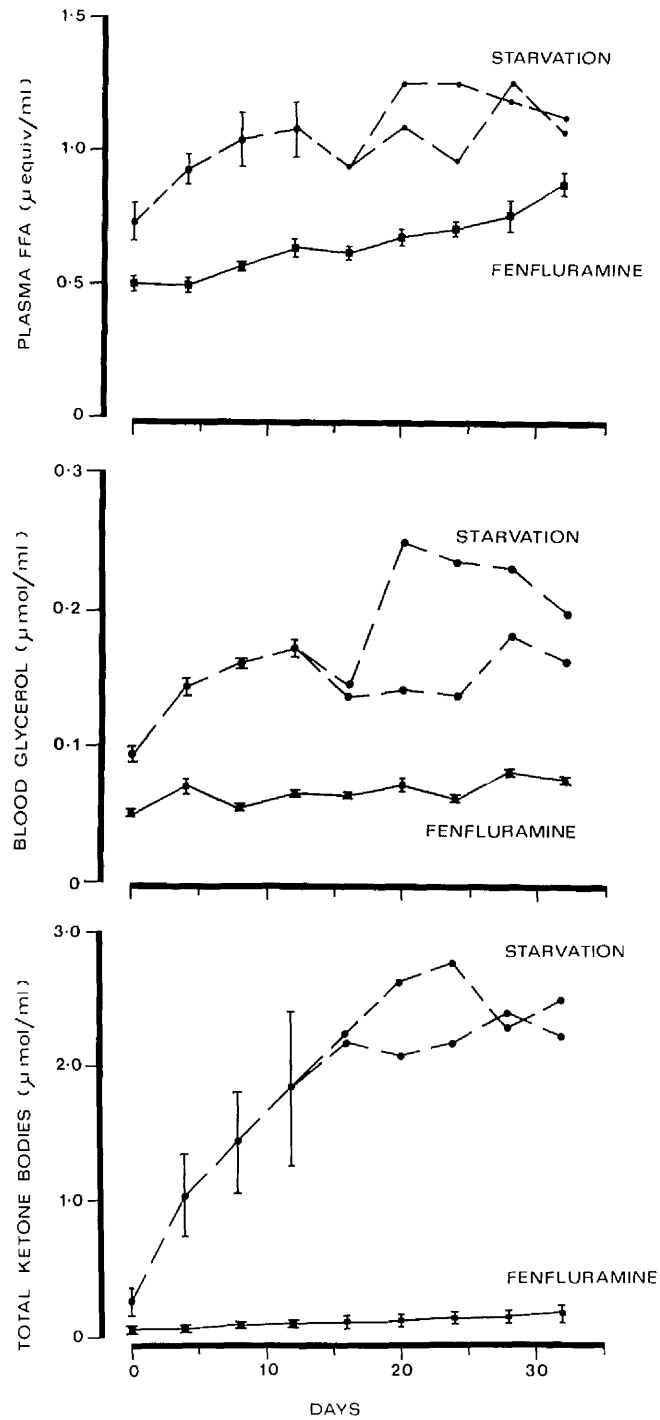
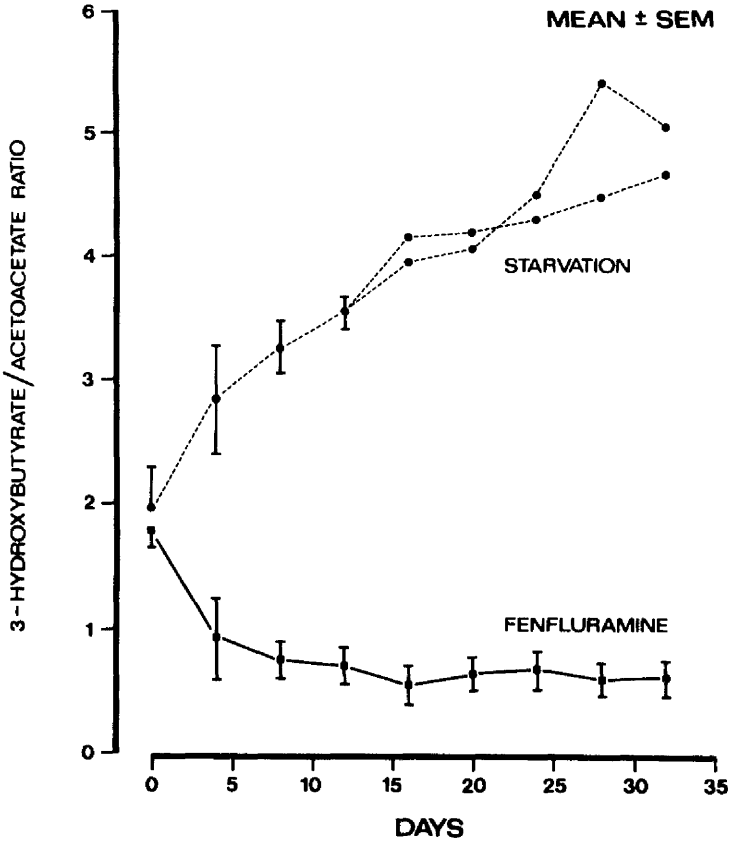

PLASMA FFA (μequiv/ml)
1·5
1·0
0·5
0
STARVATION
FENFLURAMINE
BLOOD GLYCEROL (μmol/ml)
0·3
0·2
0·1
0
STARVATION
FENFLURAMINE
TOTAL KETONE BODIES (μmol/ml)
3·0
2·0
1·0
0
STARVATION
FENFLURAMINE
0
10
20
30
DAYS

Fig (7.2): 3-hydroxybutyrate/acetoacetate ratios (mean $\pm$ SEM) in the six obese subjects treated with fenfluramine (■) and four obese subjects treated with dietary restriction (●). (see legend of Fig 7.1 for further details).


MEAN ± SEM
3-HYDROXYBUTYRATE/ACETOACETATE RATIO
STARVATION
FENFLURAMINE
DAYS
0
1
2
3
4
5
6
0
5
10
15
20
25
30
35

The concentrations of ketone-bodies (acetoacetate + 3-hydroxybutyrate, Fig 7.1) became greatly elevated in the subjects undergoing dietary restriction whereas there were much smaller increases in those receiving fenfluramine. The initial ratios of 3-hydroxybutyrate/acetoacetate were not significantly different between the two groups ($p > 0.05$). There was, however, a marked difference in the subsequent changes: the ratio increased in those subjects who underwent dietary restriction, whereas the ratio decreased in those receiving fenfluramine (Fig 7.2). Glucose concentrations fell in the subjects undergoing total starvation. During the first week, however, when the ketone-body difference became apparent, there was no statistical difference in glucose, lactate or pyruvate concentrations between the two groups.

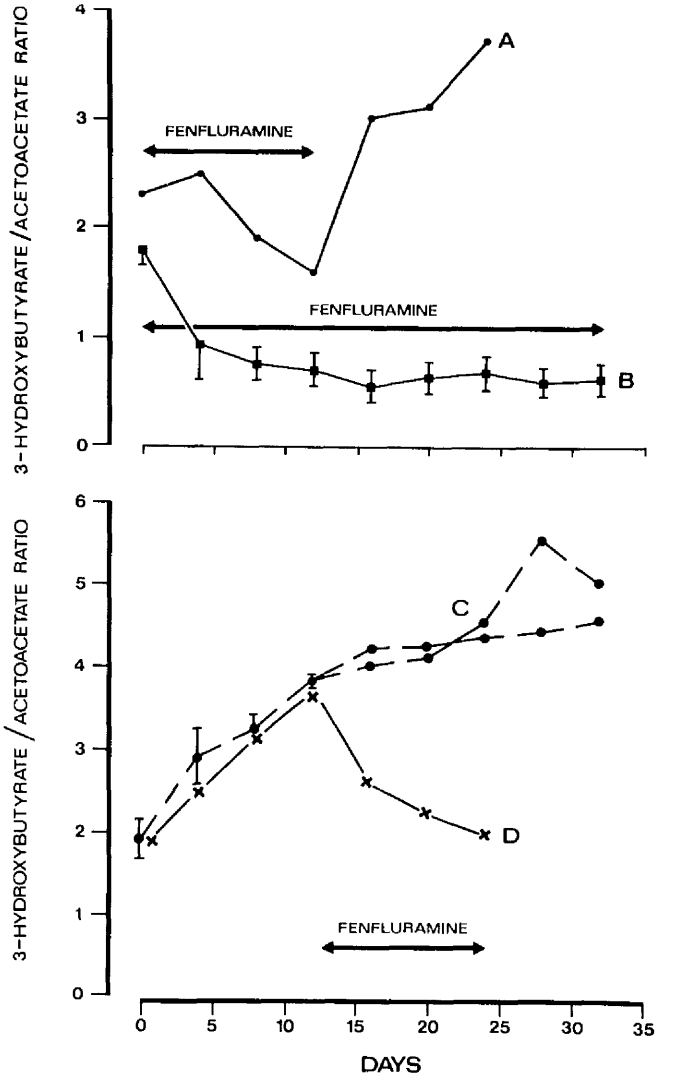
Subjects treated with fenfluramine and reduced diet

The observations in two patients treated with a combination of dietary restriction together with fenfluramine were in accord with the findings in the groups treated by either dietary restriction or fenfluramine alone. In the patient who had a 600 calorie diet and then took fenfluramine, the ratio of 3-hydroxybutyrate/acetoacetate concentrations initially increased and was depressed during the period he took the drug (Fig 7.3, D). In the other patient who was also treated with a 600 calorie diet but took fenfluramine for the first 12 days, the ratio was initially depressed but rose above resting values when fenfluramine therapy was stopped and the dietary restriction was continued alone (Fig 7.3, A). The glucose concentrations did not appear to be affected by fenfluramine. No consistent changes occurred in lactate or pyruvate concentrations in either patient.

Fig (7.3):

top, 3-hydroxybutyrate/acetoacetate ratios (A) in one patient (S.T.) who underwent restricted diet for 24 days with fenfluramine therapy in the first 12 days (⊛), (B) and in six subjects with fenfluramine (from Fig 7.2: ■).

bottom, 3-hydroxybutyrate/acetoacetate ratios (C) in four subjects treated with dietary restriction (from Fig 7.2: ●) and (D) in one patient (C.McH) who underwent restricted diet for 24 days with fenfluramine therapy in the second half of the period (X).


3-HYDROXYBUTYRATE/ACETOACETATE RATIO
FENFLURAMINE
A
FENFLURAMINE
B
3-HYDROXYBUTYRATE /ACETOACETATE RATIO
C
D
FENFLURAMINE
DAYS

DISCUSSION

Several trials have indicated that fenfluramine is of value in the treatment of obesity (Munro et al., 1966). The introduction to this thesis explained that this work began because of curiosity about the action of fenfluramine for, although widely used, the rationale remained unknown. The studies reported in this chapter were designed to investigate whether the metabolic effects of the drug are related to reduction of food intake or whether there is a direct effect of fenfluramine on pathways of fat metabolism.

In starvation there is an increase in the concentration of plasma FFA, probably due to increased lipolysis. During fenfluramine therapy in our investigation there was a rise in the plasma concentration of FFA by 60% (Fig 7.1). The increase may have been due to a decrease in the rate of re-esterification of fat as fenfluramine inhibits the metabolism of glucose to α-glycerophosphate in adipose tissue and decreases fat synthesis, perhaps by interference with the acylation of glycerol-3-phosphate at the acyl transfer reaction (Dannenburg and Kardian, 1967; Wilson and Galton, 1971). The higher concentrations of ketone-bodies in the subjects undergoing dietary restriction compared with those taking fenfluramine reflects the differences in their plasma FFA concentrations.

Despite the raised concentrations of total ketone-bodies in the blood in all the subjects during the investigation, the ratio of 3-hydroxybutyrate/acetoacetate decreased in those taking fenfluramine whereas the ratio increased in the starved subjects. An increase in the ratio is the usual change with dietary restriction, and occurs because of

a fall in a ratio of oxidised and reduced nicotinamide-adenine dinucleotide (NAD/NADH), since oxidation of fatty acids results in the production of NADH. NADH increases the catalytic conversion of acetoacetate to 3-hydroxybutyrate (Williamson et al., 1967). The depression of the ratio by fenfluramine is therefore unlikely to be related to a fall in NAD/NADH as in both groups fatty acid oxidation was occurring as adduced from the elevated ketone-body values. This would change the ratios in the opposite direction to that observed. A decrease in the ratio of 3-hydroxybutyrate/acetoacetate would occur if the peripheral utilization of acetoacetate was decreased. However, this explanation is also unlikely as the rise in ketone-bodies was very small with fenfluramine and the effect on the ratio was also produced by giving fenfluramine during starvation when the rise in ketone-bodies was much greater. Another explanation for the change in the ratio is that the drug causes decreased activity of the enzyme 3-hydroxybutyrate dehydrogenase in the liver. This may result from reduced synthesis of lecithin, an obligatory co-factor for the enzyme (Gotterer, 1967). Lecithin synthesis depends on an adequate supply of α-glycerophosphate, the production of which is affected by fenfluramine (Wilson and Galton, 1971).

It is possible that the increases in FFA and total ketone-bodies observed in the group treated with fenfluramine were due to an effect of partial starvation, because of a reduction in appetite due to the drug. However, the differences observed in the 3-hydroxybutyrate/acetoacetate ratio in this group compared with the patients undergoing dietary restriction indicate that fenfluramine is an active compound affecting metabolic pathways of fat metabolism. The relationship of

these changes to the causation of fat mobilization with fenfluramine still remained to be explored and observations upon the relationship of growth hormone to these changes are reported in the next three chapters.

SUMMARY

1. Five subjects taking fenfluramine daily (40-80 mg) for five weeks and four subjects undergoing dietary restriction for the same period without drug therapy, were investigated. Venous blood samples were taken for estimation of FFA, glycerol, ketone-bodies, lactate, pyruvate and glucose.

2. In both groups the level of FFA, glycerol and ketone-bodies rose, but to a much greater extent in the group undergoing dietary restriction. The ratio of 3-hydroxybutyrate/acetoacetate increased in this group while in those receiving fenfluramine the ratio decreased.

3. The differences in ketone-body level and ratios between the two groups may be related to an effect of fenfluramine upon enzymes involved in ketone-body metabolism. These observations imply a fundamental difference between the metabolic effect of fenfluramine and that of dietary restriction.

CHAPTER 8

ORAL GLUCOSE TOLERANCE TESTS DURING TREATMENT WITH FENFLURAMINE COMPARED WITH DIETARY RESTRICTION IN THE SAME SUBJECTS

INTRODUCTION

In the previous chapter it was shown that fenfluramine given with a low calorie diet causes increased concentrations of FFA and ketone-bodies in the blood. It has also been shown that weight reduction by diet or drugs such as fenfluramine is associated with an increased peripheral uptake of glucose (Butterfield et al., 1971). These changes during fenfluramine therapy raised the problem of whether these effects are the results of weight reduction caused by the lower dietary intake or by the direct effect of the drug on fat and carbohydrate metabolism. To investigate the effect of fenfluramine on carbohydrate metabolism a glucose tolerance test was carried out on three occasions. The first was before therapy. The second was after two weeks of dietary restriction, and the third was after two weeks on both dietary restriction and fenfluramine treatment. The investigation was carried out in a continuous four weeks period.

METHODS

Subjects: Five patients with obesity were investigated in this study (table 8.1). All the subjects were studied for four weeks, with the exception of W.R. and H.C. who completed the control investigation only.

Procedure: The subjects were admitted to hospital for the whole investigation. Initially they were brought to the laboratory and an oral glucose tolerance test (50 g) was performed. The subjects were then given a low calorie diet (600 calories) for four weeks. Fenfluramine in

Table 8.1: Individual data on age, height, weight and reciprocal ponderal index.

	Sex	Age	Height	Weight	Reciprocal ponderal index	Weight loss Kg	
		yr	cm	kg	$ht/\sqrt[3]{wt}$	Diet alone	Fenflura-mine + Diet
S.Th.	M	56	170	96.0	37.03	2.5	2.8
C.Mch.	M	51	180	92.3	39.82	2.7	2.5
J.D.	F	34	165	78.8	39.00	2.0	2.2
W.R.	M	31	168	80.6	39.63	-	-
H.C.	F	26	165	79.8	38.37	-	-

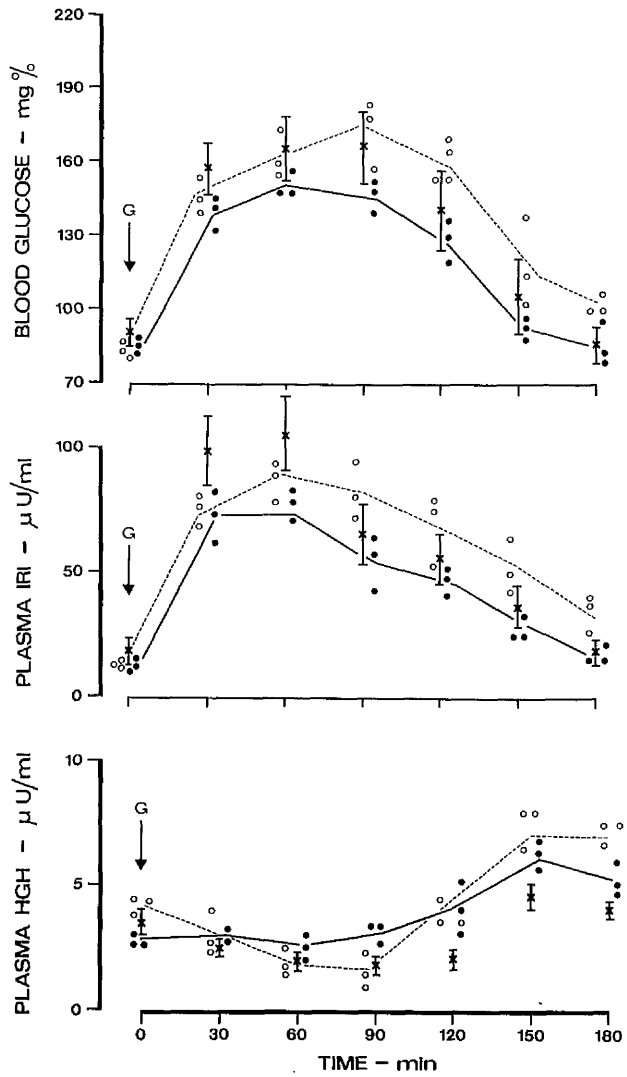
a daily dose of 100 mg was added to the diet in the last two weeks of this dietary regimen. Glucose tolerance tests were repeated before and after fenfluramine therapy under conditions similar to those of the first test. All glucose tolerance tests were performed in the morning after an overnight fast with subjects resting in bed. Several venous blood samples were taken; in the fasting state and then at $\frac{1}{2}$ hr intervals for $2\frac{1}{2}$ hr after an oral load of glucose. The samples were treated as described in appendices 1 and 2 and analysed for glucose, HGH and IRI.

RESULTS

Glucose (Fig 8.1): Fasting morning blood glucose concentrations were lower with fenfluramine and with diet alone than in the control investigation. Fenfluramine caused a marked increase of glucose tolerance so that the absolute values of blood glucose concentrations at any time throughout the test were lower than in the control study. Diet alone however, caused a decrease of glucose tolerance and the concentrations of blood glucose were considerably higher at 60, 90, 120 and 150 min during the test than in the control study.

Plasma IRI (Fig 8.1): Fasting plasma insulin concentrations during fenfluramine therapy were similar to those with reduced diet alone, and on both occasions the values were lower than on the control occasion. The rise in the levels of plasma IRI during the test paralleled the glucose concentrations, thus the levels of plasma IRI were lower throughout the test with fenfluramine. On the control occasion and with fenfluramine the maximum rise in plasma IRI occurred 60 min after glucose

Fig (8.1): The changes in the obese subjects of blood glucose (mg%), plasma IRI and HGH (μ units/ml) after ingestion of a solution of glucose (50 g) at the time indicated. Control study (5 subjects: X); reduced diet with fenfluramine (3 subjects; ⊙); reduced diet alone (3 subjects: O).


BLOOD GLUCOSE – mg %
220
190
160
130
100
70
G
PLASMA IRI – μU/ml
100
50
0
G
PLASMA HGH – μU/ml
10
5
0
G
0
30
60
90
120
150
180
TIME – min

ingestion, whereas with diet alone the maximum occurred at 90 min.

Plasma HGH (Fig 8.1): Fasting morning plasma HGH concentrations were lower with fenfluramine than in the control study. With diet alone, however, the fasting levels were higher than in the control study. In both the control investigation and with diet alone, glucose caused a depression in the concentrations of HGH at 30 min and 60 min later, but with fenfluramine, glucose caused little change. On all three occasions the concentrations rose after the depression. The values at 150 min after glucose load were higher with fenfluramine than in the control study, but they were lower than with dietary restriction alone.

DISCUSSION

The present observations are in good agreement with the recognised impairment of carbohydrate metabolism in obesity (Bortz, 1969), and showed an improvement by fenfluramine therapy. The altered carbohydrate metabolism observed in obesity and discussed in chapter 2 has been attributed to a metabolic block in peripheral uptake and oxidation of glucose by muscles (Butterfield et al., 1965). Skeletal muscle has also been found to be the primary site of insulin resistance in obesity (Rabinowitz and Zieler, 1962). The consequent hyper-insulinaemia found in obesity would facilitate fat synthesis and its deposition in adipose tissue. In this study, there is a difference in glucose tolerance between the therapy with fenfluramine and diet alone, although the loss of weight was similar on both occasions. With fenfluramine, there was an increase in glucose tolerance, whereas with diet alone the tolerance

was decreased at 90, 120 and 150 min. Similar changes have been found with fenfluramine by Asmal and co-workers (1971), in their study of the effect of weight reduction on carbohydrate metabolism. They suggested that weight reduction by both fenfluramine and diet improve the peripheral uptake of glucose. The present observation on diet alone, however, shows a decrease in glucose tolerance, which may indicate that weight reduction by fenfluramine is unlikely to be the main cause of the improvement of glucose tolerance. Furthermore, the small degree of mean weight loss (2.6%) does not parallel the marked increase in glucose tolerance with fenfluramine. Therefore fenfluramine may produce direct metabolic changes and hence cause an increased glucose tolerance. Several processes could have been affected by fenfluramine and so alter the tissue response. These could have included a greater rise of plasma insulin in response to the glucose load and increased sensitivity of glucose transport mechanism to insulin. A further possibility is that the capacity of the cells to fix insulin may be altered.

In this study however, the rise of plasma insulin in response to glucose was reduced by fenfluramine, whereas with diet alone, the rise was delayed even though the response was similar in the early part of the test on both occasions. These findings suggest that fenfluramine improves glucose tolerance by increasing the sensitivity of the peripheral muscles to insulin. It is also possible that decreased glucose tolerance with dietary restriction could be caused by the rise in the concentrations of FFA and HGH which are known to result in a decrease of insulin sensitivity (Stein et al., 1962; Garland and Randle, 1964). With fenfluramine the fasting concentrations of plasma FFA were lower than on diet alone perhaps due to the drug inhibitory effect on lipolysis which

has been experimentally stimulated by nor-adrenaline and growth hormone (Dannenburg and Kardian, 1969). The finding reported in this chapter of increased sensitivity of tissues to insulin during fenfluramine therapy raises the possibility that the drug might be active hypoglycaemic agent in diabetes mellitus. The action of fenfluramine in diabetes was therefore studied and the results are reported in the next chapter.

SUMMARY

1. Five patients with obesity undergoing dietary restriction with and without fenfluramine were studied. The subjects were given a reduced diet of 600 calories for four weeks. Fenfluramine in a daily dose of 100 mg was then added to diet therapy in the last two weeks of the study. Glucose tolerance tests were performed before starting the therapy and after each two weeks, i.e. before and after fenfluramine. Venous blood samples were taken for the estimation of blood glucose, plasma IRI and HGH.

2. Fenfluramine increased glucose tolerance and reduced the insulin response during an oral glucose tolerance test. Reduced diet alone however, caused a reduction in glucose tolerance and a delay in the rise of insulin response to the glucose load.

3. The hypoglycemic effect of fenfluramine therefore appears to be due, at least in part to a direct effect of the drug increasing the tissue sensitivity to insulin.

CHAPTER 9

SINGLE ADMINISTRATION OF FENFLURAMINE IN NORMAL SUBJECTS AND IN PATIENTS WITH DIABETES MELLITUS

INTRODUCTION

In the previous chapters it was shown that fenfluramine given with a low calorie diet causes concentrations of plasma FFA and blood ketone-bodies to rise. Increased glucose tolerance and reduced insulin response to glucose load were also observed with fenfluramine and low calorie diet. They imply that the metabolic changes with fenfluramine include a direct effect which is not necessarily secondary to dietary restriction. These changes might have been due to the drug's action on pathways in peripheral tissue regulating glucose transport and insulin metabolism. Since most of the metabolic processes which are known to be altered by fenfluramine are influenced by hormones, it is also possible that the beneficial effects of this drug are mediated through alteration of endocrine activity and studies of the effect of the drug on growth hormone release are given in the next chapter. It is the purpose of the present study to examine fat and carbohydrate metabolism in relation to plasma HGH and IRI after acute administration of fenfluramine in normal subjects and the results showed that fenfluramine causes a slight fall in the concentration of plasma HGH. Abnormalities of fat and carbohydrate metabolism associated with high levels of HGH have been reported in diabetes mellitus (Randle et al., 1963). The plasma HGH level fluctuates in this disorder, but is frequently very high for long periods of time (Lundbaek, 1971). The hyper-secretion of HGH may lead to the development of angiopathy in some cases of diabetes which may lead to severe retinal changes. Attempts to suppress growth hormone release in these patients has been made by pituitary extirpation (hypophysectomy) as the use of various pharmacological agents have failed to give satisfactory results (Hansen, 1971).

An opportunity to examine further the effect of fenfluramine on HGH release in relation to fat and carbohydrate metabolism is provided by the study of patients with diabetes. I have therefore studied a group of patients with diabetes. They were given fenfluramine intravenously at rest to determine the way in which their metabolic responses to the drug differ from those of normal subjects. The changes in the patterns of blood metabolites which resulted are discussed in relation to the drug actions on HGH and IRI release.

METHODS

Effect of acute injection of fenfluramine in normal subjects: Seven healthy volunteers (5 male and 2 female, aged 24-54 yr) with mean ht of 164 cm and mean wt of 78.2 Kg were studied. They were not receiving any drugs at the time of the investigation and were eating a normal diet. The subjects were brought to the laboratory between 09.00 - 10.00 hr after overnight fast. A catheter was placed in an antecubital vein and intravenous fenfluramine (20 mg) was administered. Blood samples were taken before the injection and 5, 10, 20, 30, 45, 60, 90 and 120 min later.

Effect of acute injection of fenfluramine on patients with diabetes mellitus: Five female patients with diabetes (aged 43-60 yr) with mean ht of 158 cm and mean wt of 83 Kg were studied in the same manner as described above. Their mean blood glucose concentrations after overnight fasting was 160 mg/100 ml ($\pm$ 12 mg SEM) and they were studied while investigated for diabetes when no therapy had been started. The blood samples were treated as described in appendices I and II, and were analysed for metabolites and hormones.

RESULTS

Effect of acute injection of fenfluramine in normal subjects

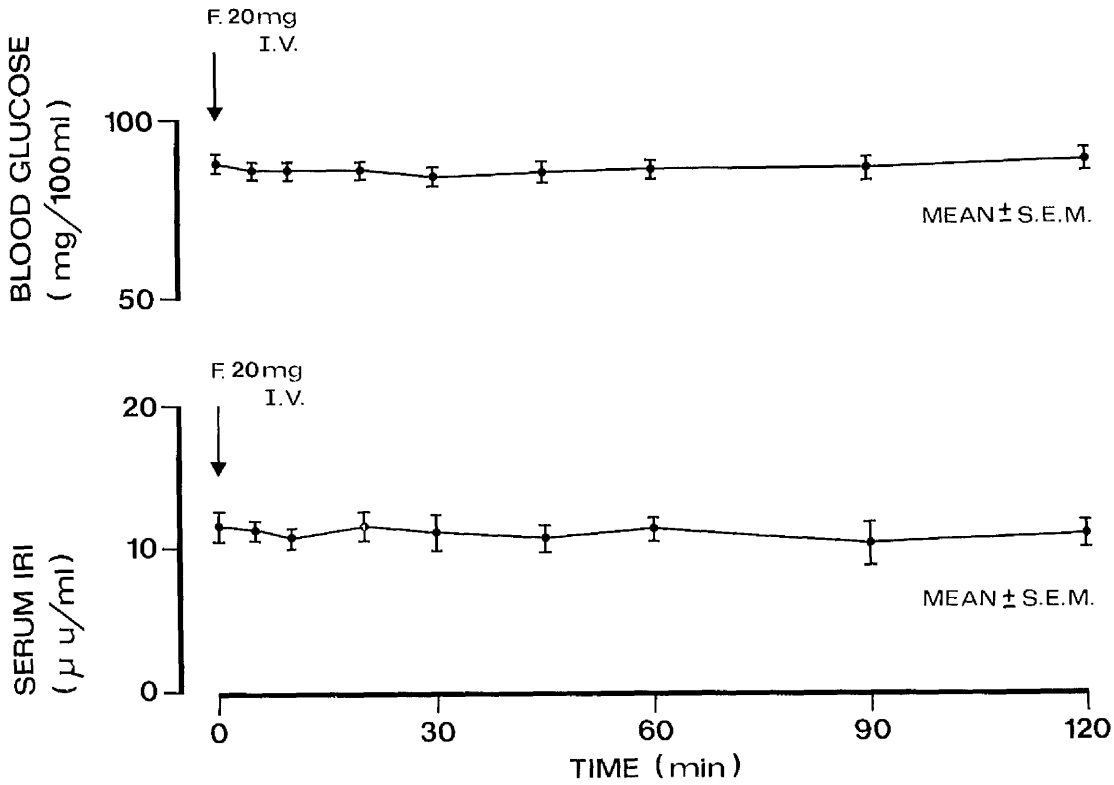
Blood glucose and plasma IRI (Fig. 9.1): The initial concentrations of both blood glucose and plasma IRI were within the normal range and remained unchanged by fenfluramine throughout the period of investigation.

Plasma FFA and blood glycerol (Fig. 9.2): Before the injection of fenfluramine the concentrations of plasma FFA and blood glycerol were 0.56 μ equiv/ml (± 0.04 μ equiv SEM) and 0.048 μ mol/ml (± 0.006 μ mol SEM) respectively. After fenfluramine there was a rise in the concentration of FFA which was first observed 60 min after the injection of the drug. The maximum rise occurred at the end of the investigation. FFA values at 60, 90 and 120 min were significantly different from rest ($p < 0.05$, 0.01 and 0.01), blood glycerol concentrations, however, were not significantly altered by fenfluramine, although they fell slightly in the early part of the investigation. Increased ratios of FFA/glycerol were observed at 60, 90 and 120 min after fenfluramine injection.

Total ketone-bodies (Fig. 9.3): Fenfluramine caused a rise in the concentrations of total ketone-bodies which was first observed 60 min after the injection. The maximum at the end of the investigation was significantly different from those before the injection ($p < 0.01$). The ratio of 3-hydroxybutyrate/acetoacetate was decreased from 2.06 (± 0.13) to 0.56 (± 0.06) 5 min after the injection. Decreased ratios were maintained for a further 25 min, after which a progressive rise towards the initial values was observed. The value at the end of the investigation was slightly lower but not significantly different from that before the injection.

Fig (9.1): Blood glucose (mg%) and plasma IRI (μU/ml; mean ± SEM) in seven healthy subjects after administration of fenfluramine (20 mg i.v.).

Fig (9.2): Blood glycerol (μ mol/ml), plasma FFA (μ equiv/ml) and FFA/Glycerol ratio; mean ± SEM in seven healthy subjects after administration of fenfluramine (20 mg i.v.).


F. 20mg
I.V.
BLOOD GLUCOSE
(mg/100ml)
100
50
MEAN ± S.E.M.
F. 20mg
I.V.
SERUM IRI
(μ u/ml)
20
10
0
MEAN ± S.E.M.
0
30
60
90
120
TIME (min)

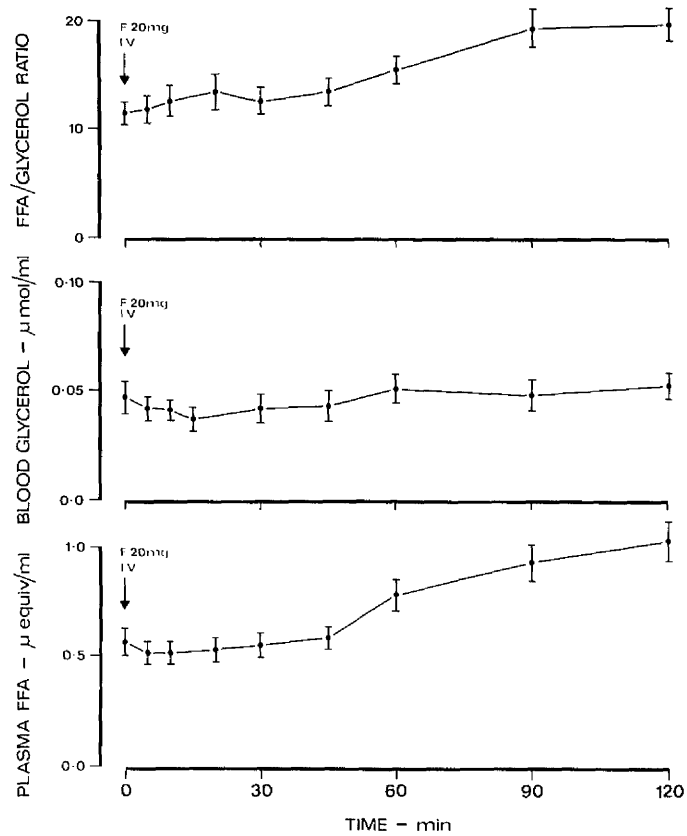
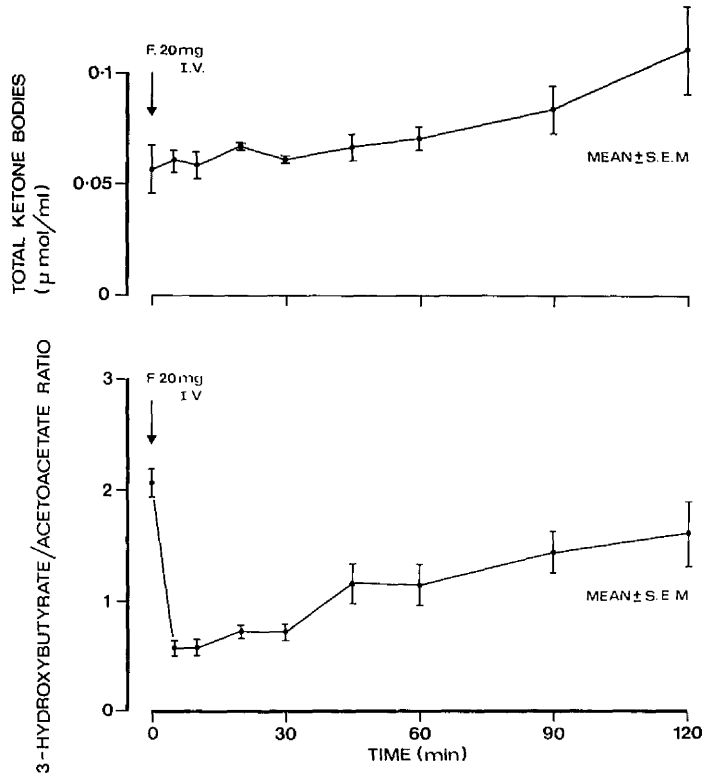
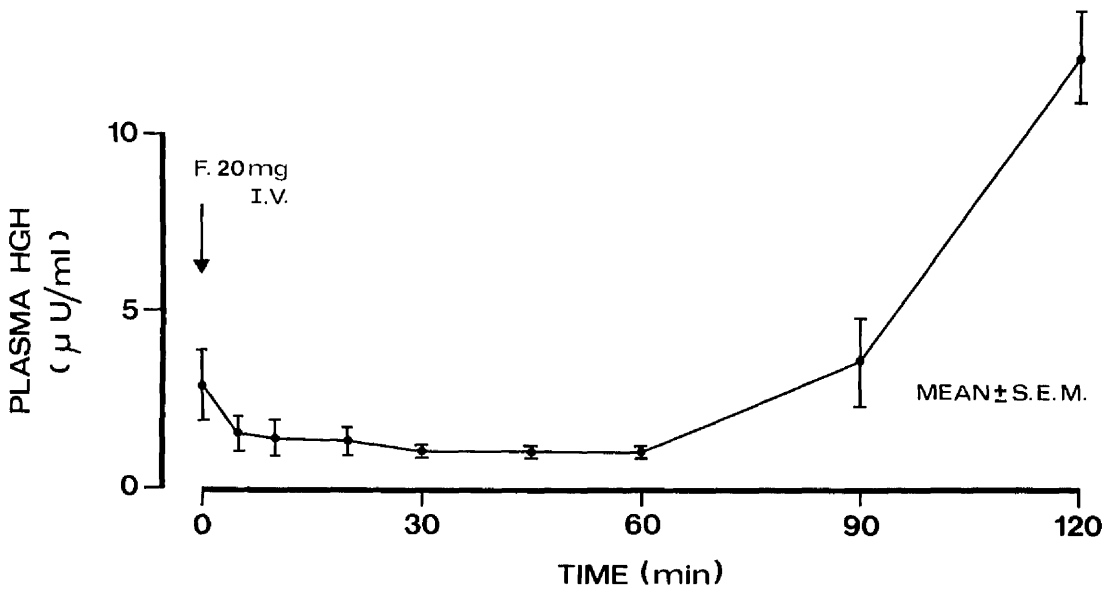

FFA/GLYCEROL RATIO
20
10
0
F 20mg
IV
BLOOD GLYCEROL – μ mol/ml
0·10
0·05
0·0
F 20mg
IV
PLASMA FFA – μ equiv/ml
1·0
0·5
0·0
F 20mg
IV
0
30
60
90
120
TIME – min

Fig (9.3): Blood ketone-bodies (μ mol/ml) and the ratio of 3-hydroxybutyrate/acetoacetate: mean ± SEM in seven healthy subjects after administration of fenfluramine (20 mg i.v.).

Fig (9.4): Plasma HGH (μU/ml; mean ± SEM) in seven healthy subjects after administration of fenfluramine (20 mg i.v.).


F. 20mg
I.V.
0·1
0·05
0
TOTAL KETONE BODIES
(μ mol/ml)
MEAN±S.E.M
F. 20mg
I.V.
3
2
1
0
3-HYDROXYBUTYRATE/ACETOACETATE RATIO
MEAN±S.E.M
0
30
60
90
120
TIME (min)


10
5
0
PLASMA HGH
(μU/ml)
F. 20mg
I.V.
MEAN±S.E.M.
0
30
60
90
120
TIME (min)

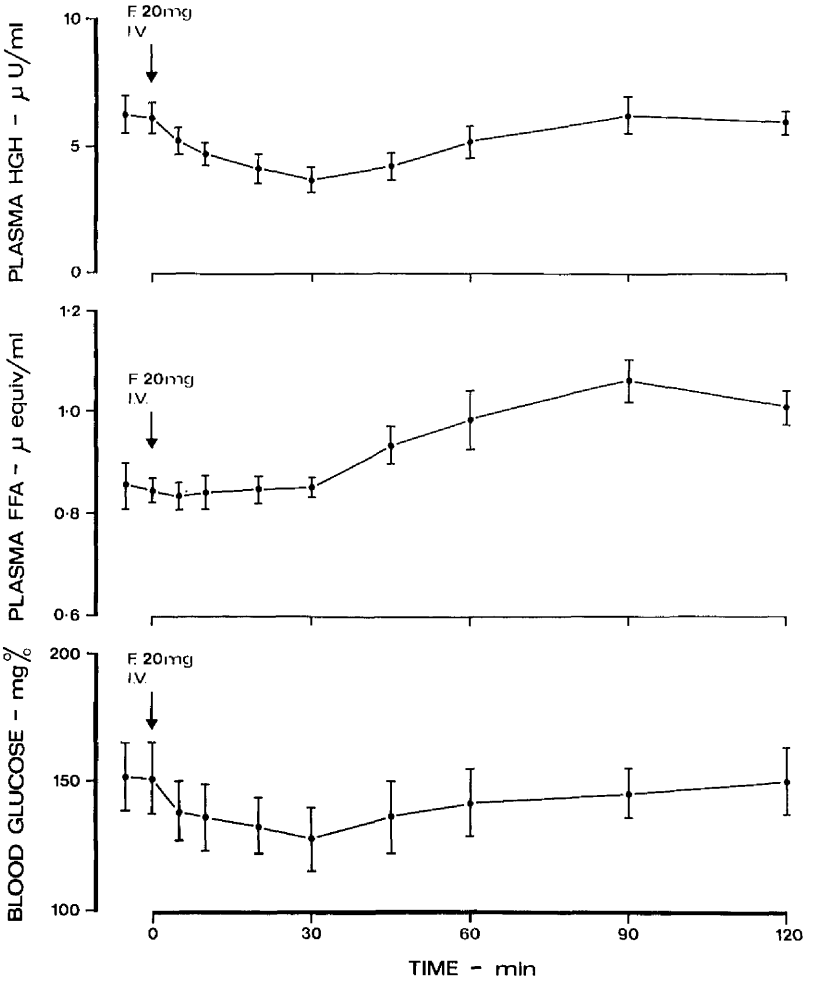
Plasma HGH (Fig 9.4): The initial concentration of plasma HGH was 2.8 μU/ml (± 0.5 μU SEM). Fenfluramine caused an immediate fall in the concentration to a minimum at 30 min. HGH concentration returned to pre-injection value at 60 min and then rose.

Effect of acute injection of fenfluramine on patients with diabetes mellitus

Blood glucose (Fig 9.5): Before the injection of fenfluramine the concentration of blood glucose was 160 mg % (± 12 mg SEM). After fenfluramine there was a fall in the concentration of glucose which was first observed 5 min after the administration of the drug. The minimum values at 45 min were significantly different from those before the injection of fenfluramine ($P < 0.01$). The concentrations then rose to achieve pre-injection values 30 min later. Plasma IRI concentrations however were not significantly altered by fenfluramine.

Plasma FFA and HGH (Fig 9.5): The initial concentration of FFA was 1.05 μ equiv/ml (± 0.02 μ equiv SEM). Fenfluramine caused a rise in the concentrations of FFA which was first observed 45 min after the injection. The maximum rise occurred at 90 min. The values at 45, 60 and 90 min were significantly higher than those before the injection ($P < 0.05$). Plasma HGH concentrations before the injection of fenfluramine were 7.8 μ U/ml (± 2.3 μ U SEM). The drug caused the concentrations of plasma HGH to fall immediately after the injection to reach a minimum at 30 min. The values at 20 and 30 min were significantly lower than the initial value ($P < 0.01$). HGH concentration then returned to pre-injection value within 60 min after the injection of fenfluramine.

Fig (9.5): Blood glucose (mg%), plasma FFA (μ equiv/ml) and HGH (μU/ml; mean ± SEM) in five diabetic patients after administration of fenfluramine (20 mg i.v.).


F 20mg
IV
PLASMA HGH - μ U/ml
10
5
0
F 20mg
IV
PLASMA FFA - μ equiv/ml
1·2
1·0
0·8
0·6
F 20mg
IV
BLOOD GLUCOSE - mg%
200
150
100
0
30
60
90
120
TIME - min

DISCUSSION

In this study of the effects of intravenous administration of fenfluramine to normal subjects, the drug caused a delayed rise in both FFA and ketone-body concentrations. FFA release would occur if fenfluramine stimulates fat mobilization from adipose tissue, but this is unlikely since the rise of FFA was not accompanied by a parallel rise in blood glycerol. Another explanation for the rise in the concentration of FFA is a decreased rate of fat re-esterification in adipose tissue. This may result from reduced synthesis of α-glycerophosphate from glucose, the oxidation of which is reduced by fenfluramine in adipose tissue (Daenenburg and Kardian, 1969). Furthermore fenfluramine has been shown to interfere with the acylation reaction and leads to the accumulation of long chain acyl CoA in liver hemogenates (Wilson and Galton, 1971). Such a suggestion is consistent with the findings reported in chapter 7. The rise in the values of ketone-bodies concentrations is probably secondary to the rise in the concentration of FFA and their subsequent oxidation in the liver. Increased rate of ketone-body production is always accompanied by elevation of the ratio of 3-hydroxybutyrate/acetoacetate, probably as a result of decreased ratio of NAD/NADH at the site of 3-hydroxybutyrate dehydrogenase within the mitochondria, as in starvation. With fenfluramine however, the ratio was depressed, mostly within 5 min of the injection of fenfluramine, and a low value was maintained to the end of the investigation. This also is compatible with the previous finding reported in chapter 7 in which it was suggested that fenfluramine affects enzymes regulating ketone-body metabolism. The most striking finding was the effect of fenfluramine on HGH concentrations in the plasma.

Although the initial concentrations of HGH were low, they fell slightly in the first five minutes after the injection of fenfluramine. This may be due to a central depressive action on centres in the hypothalamus regulating the release of HGH, as fenfluramine is known to have an effect on centres in the hypothalamus regulating appetite (Foxwell et al., 1968). It was surprising that the drug had no effect on fasting blood glucose and plasma IRI concentrations in normal subjects, but when it was given to patients with diabetes mellitus a marked fall in the concentration of blood glucose was observed. This fall disappeared as soon as there was a rise in plasma FFA concentration. Subsequent to carrying out this investigation the observations of Turtle and Burgess (1973) have been reported and the results are consistent. Blood glucose depression may have been due to the drug's action upon peripheral uptake of glucose (Butterfield and Whichelow, 1968). The action was, however, of short duration and another explanation could be dependent on FFA concentrations as FFA are known to interfere with glucose metabolism (Garland and Randle, 1964). In the patients with diabetes mellitus fenfluramine also caused a marked fall in plasma HGH concentration similar to the changes observed in normal subjects. This is consistent with the suggestion that fenfluramine may interfere with pathways in the hypothalamus regulating the release of HGH. The possible importance of HGH in diabetes mellitus has already been discussed in the introduction to this chapter. It was explained that the high concentrations of HGH found in diabetes may be causative in the development of blood vessel pathology.

The findings reported in this chapter of both glucose and growth hormone suppression by fenfluramine suggest that the drug may be of

value in clinical management of diabetes mellitus.

A basic problem in the observations upon HGH reported in this chapter is that at rest plasma HGH concentrations are low in normal subjects (2-6 μU/ml). In diabetes mellitus concentrations are variable although higher values are observed (6-10 μU/ml). The action of fenfluramine on HGH release was therefore studied during exercise in normal subjects, as release of HGH usually occurs during exercise. These observations together with studies on the action of fenfluramine on HGH release in acromegaly are reported in the next chapter.

SUMMARY

NORMAL SUBJECTS

1. The effects of acute administration of fenfluramine were studied at rest in seven healthy volunteers. Fenfluramine (20 mg) was given intravenously to all the subjects after an overnight fast.

2. Fenfluramine caused a fall in the concentrations of plasma HGH immediately after the injection and for 30 min afterwards. The drug also affected plasma FFA and blood ketone-bodies concentrations.

DIABETES MELLITUS

3. The effect of fenfluramine was observed in five patients with diabetes mellitus. A fall in the concentration of HGH was also seen in the diabetics. Their blood glucose concentrations were lowered by fenfluramine, but returned to pre-injection values soon after the release of FFA.

4. These observations suggest that fenfluramine has in addition to a peripheral action on fat and carbohydrate metabolism, a central action affecting the release of HGH. These effects were more marked in the diabetics suggesting that the drug should be evaluated in the medical management of diabetes mellitus.

CHAPTER 10

EXERCISE AFTER ACUTE ADMINISTRATION OF FENFLURAMINE

INTRODUCTION

When the acute effect of fenfluramine was studied in normal individuals and in patients with diabetes, changes in the concentrations of HGH were observed. Although the initial concentrations of HGH were low, fenfluramine caused a depression in the levels of plasma HGH when subjects were at rest (chapter 9). This effect of fenfluramine might have been due to a depressant action on centres in the central nervous system regulating appetite and food intake (Foxwell et al., 1968). Experimental observations on animals have indicated the hypothalamus as a possible site for the action of the drug (Anand, 1971; Anand and Blundell, 1971). The hypothalamus influences the secretion of hormones of the anterior pituitary, including growth hormone, via intermediary neurohormones (McCann, 1970). It is therefore possible that alteration of release of growth hormone may occur with drugs affecting the hypothalamus. The resting levels of HGH are however very low in normal individuals (2-6 μU/ml) and I therefore decided to observe the action of the drug in conditions in which HGH concentrations are raised, and chose exercise and acromegaly. Exercise is a situation known to stimulate the release of HGH (Hunter and Greenwood, 1964; Hunter et al., 1965), and I have studied the effect of fenfluramine on the release of HGH and insulin in exercise in normal volunteers.

Hypothalamic dysfunction may result in acromegaly in some individuals. In acromegaly the hypersecretion of HGH is not always autonomous (Cryer and Daughaday, 1969; Lawrence et al., 1970) and the level may rise with an appropriate stimulus such as exercise (Johnson and Kennie, 1973). I have therefore studied a group of patients with

acromegaly when given fenfluramine. One patient was also exercised after being given the drug. The present observations in normal subjects and patients with acromegaly indicate the suppressive action of fenfluramine on HGH release.

METHODS

The effect of acute injection of fenfluramine in normal volunteers

Five healthy male volunteers (aged 24-38 yr) with mean ht of 174 cm and mean wt of 78.2 kg, exercised on a bicycle ergometer on two separate occasions. On both occasions the subjects had fasted overnight, and exercised for 20 min at 600 kpm, followed by a period of rest of 90 min. Blood samples were taken from a catheter in an antecubital vein. Two resting samples were taken, further samples were also taken at 5 min intervals during exercise and then at 15, 30, 60 and 90 min after the exercise. On the second occasion fenfluramine (20 mg i.v.) was given between the resting samples and exercise was started 5 min later. Heart rate was recorded with a continuous electrocardiograph.

The effect of acute injection of fenfluramine on patients with acromegaly

(a) At rest Four patients with acromegaly (three female and one male, aged 44-72 yr) with mean ht of 161 cm and mean wt of 72.3 kg were studied. Their mean resting plasma HGH level after overnight fasting was 65.5 μU/ml ($\pm$ 14.7 μU SEM) and they were studied during investigation for acromegaly when no other therapy was being given. On the day of the investigation, the patient had fasted overnight and rested in bed. A catheter was placed in an

antecubital vein and fenfluramine (20 mg i.v.) was administered. Venous samples were taken before the injection, and 5, 10, 20, 30, 45, 60 and 90 min later.

(b) Exercise One male patient with acromegaly (aged 21 yr; ht 174 cm; wt 76 kg) was studied on two separate occasions in the same manner as described above. His resting plasma HGH level after overnight fasting was 76-74 μU/ml and no treatment or replacement therapy had been started.

The blood samples were treated as described in appendices I and II and analysed for glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, FFA, IRI and HGH.

RESULTS

The effect of acute injection of fenfluramine in normal volunteers

Lactate and pyruvate (fig 10.1) Resting blood lactate concentrations were similar on both occasions. During exercise the concentrations rose to a maximum at 15 min when there was no significant difference between the two occasions. The lactate concentrations returned to resting levels 60 min after exercise. The changes in pyruvate concentrations were similar.

Glucose (fig 10.2) There was no difference in the concentrations of blood glucose on the two occasions. Exercise caused a fall in the concentrations at 5 min in the control investigation, but with fenfluramine the fall was more marked after 15 min of exercise when the concentration was significantly different from that of the control study ($P < 0.05$). Although the levels during recovery were not statistically

Fig 10:1 Blood lactate and pyruvate levels (μ mol/ml, means ± SEM) in five subjects during and after two periods of 20 min exercise;

control period ■- - - -■ ; with fenfluramine ●————●

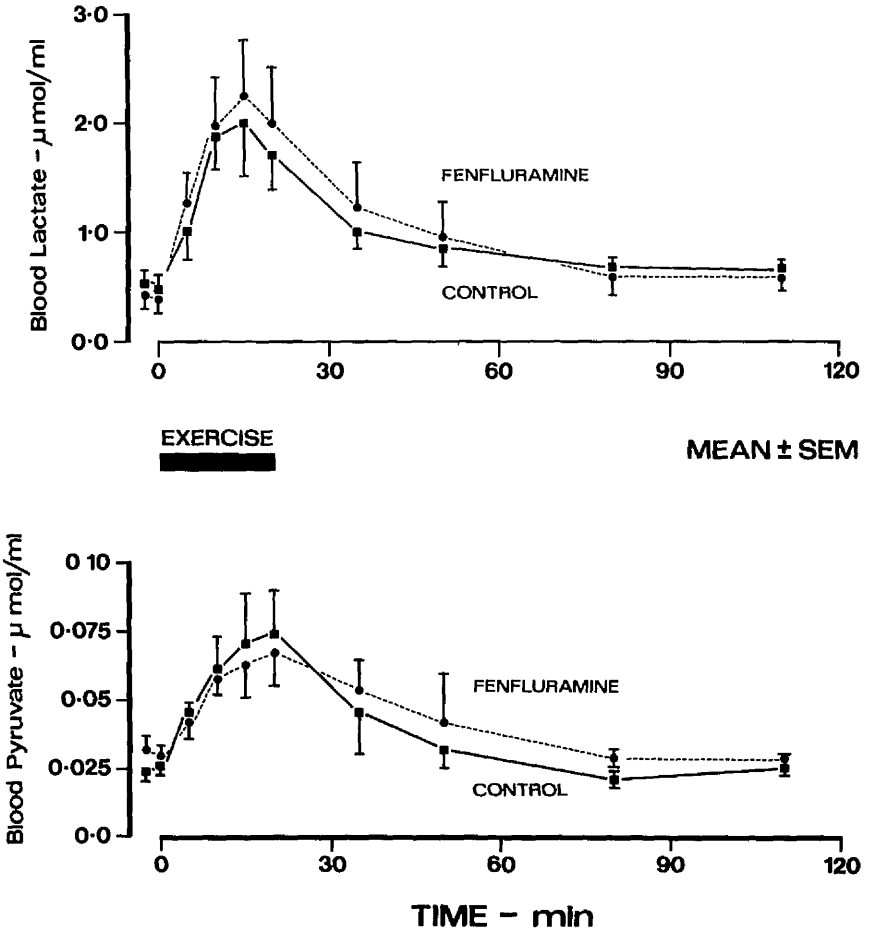
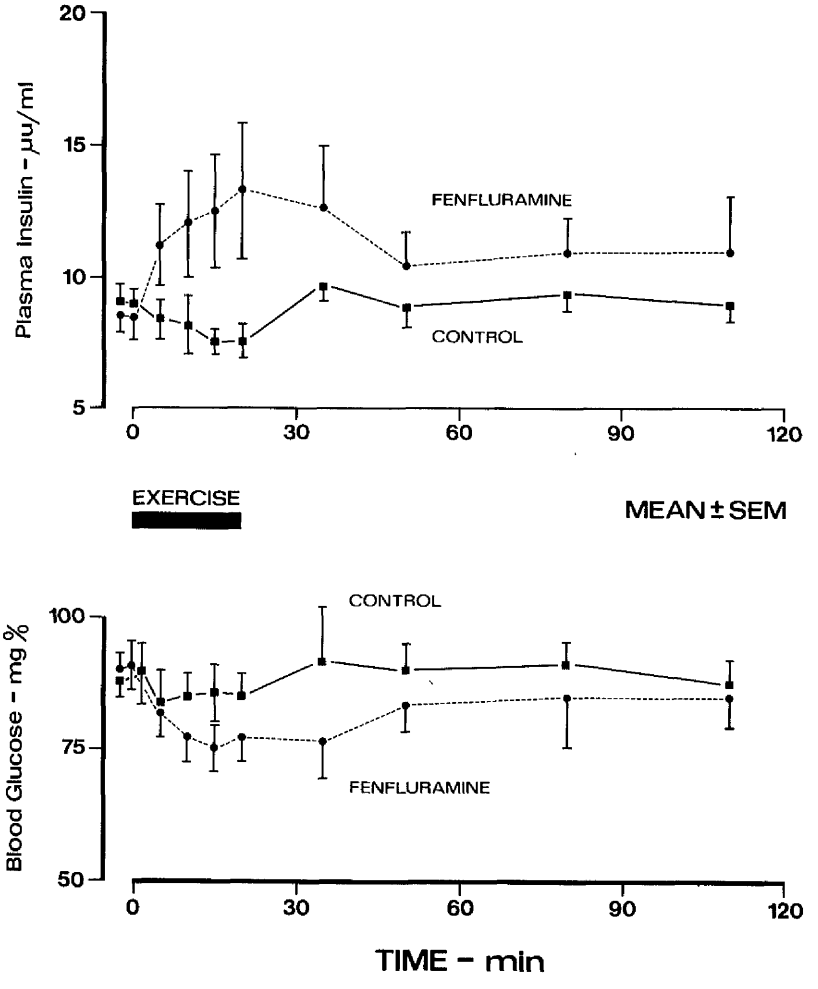

Blood Lactate - μmol/ml
3·0
2·0
1·0
0·0
FENFLURAMINE
CONTROL
0
30
60
90
120
EXERCISE
MEAN ± SEM
Blood Pyruvate - μ mol/ml
0 10
0·075
0·05
0·025
0·0
FENFLURAMINE
CONTROL
0
30
60
90
120
TIME - min

Fig 10:2 Blood glucose (mg%) and plasma IRI (μ units/ml, means ± SEM) in five subjects during and after two periods of 20 min exercise;

control period ■-----■; with fenfluramine ●——●


Plasma Insulin - μu/ml
20
15
10
5
FENFLURAMINE
CONTROL
0
30
60
90
120
EXERCISE
MEAN ± SEM
Blood Glucose - mg %
100
75
50
CONTROL
FENFLURAMINE
0
30
60
90
120
TIME - min

different at any one time on the control occasion, the concentrations were consistently higher and returned to the resting level 15 min after exercise, whereas with fenfluramine the concentrations remained below the resting level throughout the remaining period of investigation.

FFA (fig 10:3) Resting plasma FFA concentrations were not significantly different on the two occasions. Exercise caused similar rises in the concentrations of FFA with the maxima at the end of exercise. In the control investigation the concentrations returned to resting levels by the end of the investigation, whereas with fenfluramine the concentrations of FFA remained higher in the post-exercise period although they were only significantly different from that of the control study at 30 min after exercise ($P < 0.01$).

Ketone-bodies (fig 10:3) Resting levels of total ketone-bodies (acetoacetate plus 3-hydroxybutyrate) were not statistically different at rest. Exercise caused a small fall in concentrations on the control occasion, but with fenfluramine the fall was more marked ($P < 0.01$). On both occasions, the levels rose after the initial fall, both in the remaining part of exercise and for 15 min afterwards. The concentration was much greater at 15 min after exercise on the control occasion (100% of resting) than with fenfluramine (14% of resting). In the remaining period of the investigations the levels of ketone-bodies slowly returned toward the resting value.

Plasma IRI (fig 10:2) Resting concentrations of plasma IRI were not statistically different on the two occasions. The concentration fell during exercise in the control study, but rose with fenfluramine so that the difference in the concentrations between the two studies was

Fig (10.3) Plasma FFA (u equiv/ml) and blood ketone bodies (u mol/ml, means ± SEM) in five subjects during and after two periods of 20 min exercise;

control period ■---■; with fenfluramine ●——●

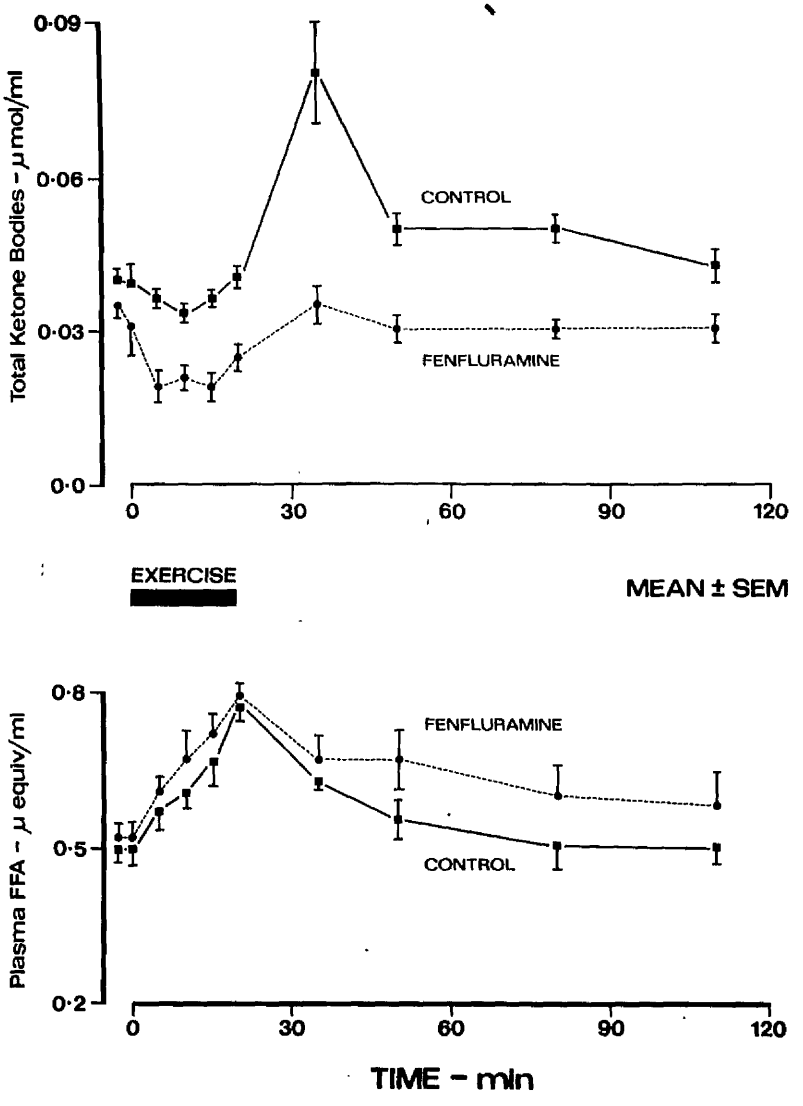

0·09
0·06
0·03
0·0
Total Ketone Bodies - µmol/ml
CONTROL
FENFLURAMINE
0
30
60
90
120
EXERCISE
MEAN ± SEM
0·8
0·5
0·2
Plasma FFA - µ equiv/ml
FENFLURAMINE
CONTROL
0
30
60
90
120
TIME - min

Fig (10.4) Plasma HGH (u units/ml, mean ± SEM) in five subjects after two periods of 20 min exercise;

control period ■——■; with fenfluramine ◎——◎

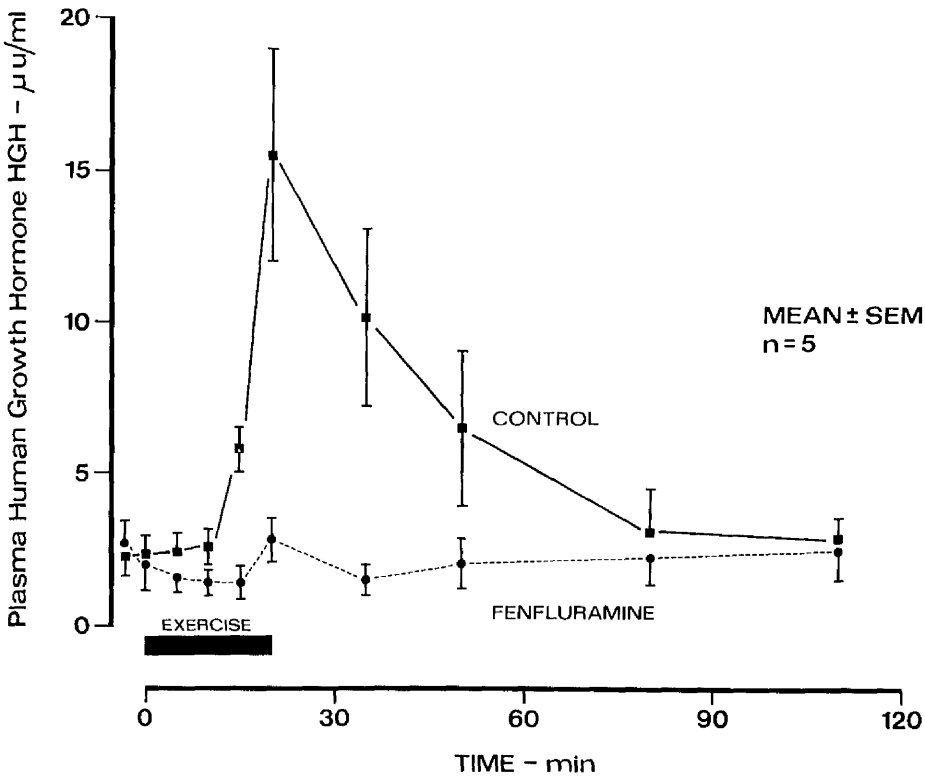
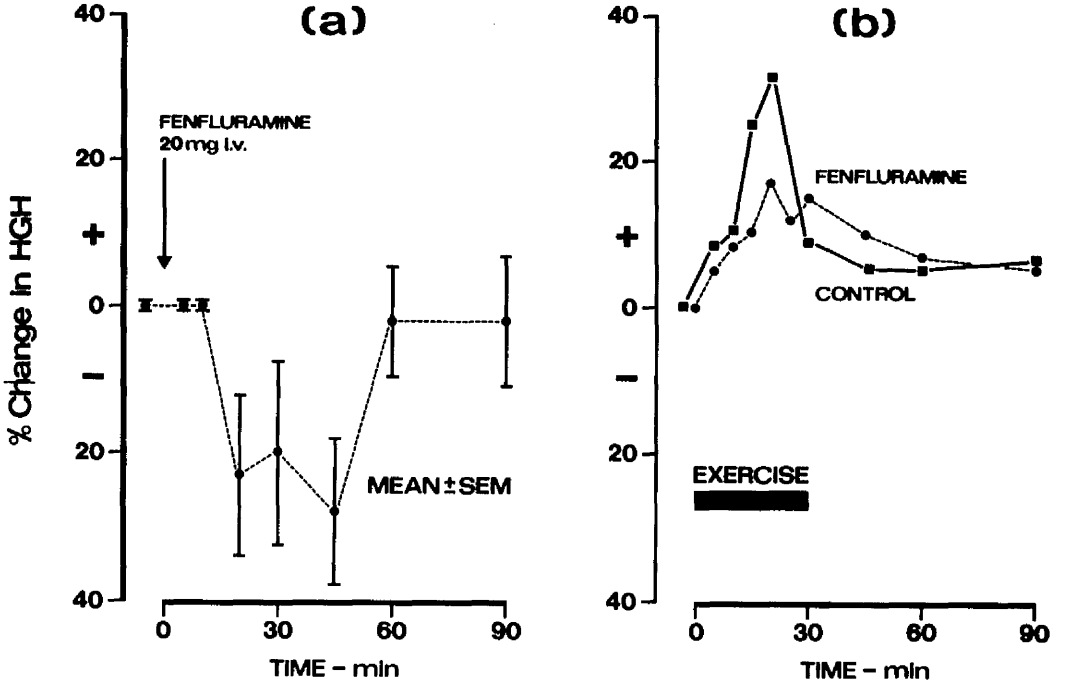

Plasma Human Growth Hormone HGH - μu/ml
20
15
10
5
0
MEAN ± SEM
n = 5
CONTROL
FENFLURAMINE
EXERCISE
0
30
60
90
120
TIME - min

Fig 10:5 Percentage change in plasma HGH (means ± SEM)

(a) in four acromegalic patients following administration of fenfluramine (20 mg i.v.) at rest

(b) in an acromegalic patient during and after two periods of 30 min exercise;

control period ■- - - - -■ ; with fenfluramine ●———● (20 mg i.v.)


(a)
40
20
+
0
−
20
40
% Change in HGH
FENFLURAMINE
20mg i.v.
MEAN±SEM
0
30
60
90
TIME – min
(b)
40
20
+
0
−
20
40
FENFLURAMINE
CONTROL
EXERCISE
0
30
60
90
TIME – min

significant throughout exercise ($P < 0.05$). In the control investigation the level of IRI returned to the resting value 15 min after exercise, but with fenfluramine the level returned more slowly and remained above the resting value.

Plasma HGH (fig 10:4) At rest plasma HGH were similar on both occasions. During exercise there was a rise of 600% in the level toward the end of exercise on the control investigation. With fenfluramine, however, the rise was abolished.

The effect of acute injection of fenfluramine on patients with acromegaly

(a) At rest (fig 10:5a) Fenfluramine caused the concentration of HGH to fall 25%, 20% and 28% 20, 30 and 45 min after the injection, respectively ($P < 0.01$). The concentration returned to the original concentration 60 min after the injection.

(b) Exercise (fig 10:5b) On the control occasion exercise caused a progressive rise of HGH to a maximum of 32% above the resting level, whereas with fenfluramine the maximum was 17% above resting level.

DISCUSSION

Exercise improves the peripheral uptake of glucose and its subsequent utilization. Increased glucose utilization also activates ketone-body utilization in exercised muscle. Fenfluramine caused a greater fall in the concentrations of blood glucose and ketone-bodies during exercise which may be attributed to increased peripheral uptake of glucose and its subsequent utilization. Increased glucose utilization by exercising muscles depends on the concentration of glycogen within

the muscle. Although I have not studied muscle glycogen content, it is unlikely that decreased glycogen concentration in the muscle is the cause of the changes with fenfluramine because lactate and pyruvate concentrations were not altered. Fenfluramine, however, caused a rise in the concentration of plasma IRI during exercise instead of the usual fall. This increase could have contributed to differences in the level of blood glucose and ketone-bodies. Despite the rise in the level of plasma IRI with fenfluramine the changes in FFA with exercise were similar on both occasions. In the investigation with fenfluramine, however, the level of FFA remained higher in the post-exercise period. This might suggest that fat mobilization is not appreciably affected by fenfluramine, although the drug may reduce the rate of fat re-esterification. This supports the observation that *in vitro* fenfluramine inhibits the metabolism of glucose to α-glycerophosphate in adipose tissue and decreases fat synthesis (Wilson and Galton, 1971).

The secretion of HGH from the anterior pituitary is influenced by the hypothalamus through an intermediary neurohormone system which is probably controlled by adrenergic mechanisms (Fuxe and Hökfelt, 1969; Wurtman, 1970; Forhman, 1972). Some drugs, such as L-DOPA (Eddy *et al.*, 1971; Kansal *et al.*, 1972) and amphetamine (Besser *et al.*, 1969), cause increased nor-adrenaline synthesis or turnover in brain and stimulate the release of HGH probably by stimulating hypothalamic activity. The cause of HGH release with exercise may therefore result from increased nor-adrenaline in the hypothalamus. Fenfluramine, however, depletes cerebral amines and decreases their concentration particularly in the hypothalamus (Costa *et al.*, 1971; Ziance *et al.*, 1972), and it may, therefore, interfere with HGH release by affecting

the adrenergic activity of the hypothalamus. This mechanism may also explain the failure of the fall in glucose concentrations to stimulate HGH production in the investigation with fenfluramine, for hypoglycaemia also acts via its effect on adrenergic mechanisms in the brain (Blackard and Keidingsfelder, 1966).

In acromegaly, HGH release may still occur in response to some stimuli such as a glucose load and exercise (Cryer and Daughaday, 1969; Lawrence et al., 1970), indicating that the hypothalamic-pituitary axis is still active in some patients. In the patients of this study fenfluramine caused a depression in the concentration of HGH at rest and the rise in HGH during exercise in one patient was attenuated by the drug. These observations suggest that the drug may be of value in the medical management of acromegaly. There are, however, other situations particularly childhood and adolescence in which the drug might have undesirable effects on growth. Short periods of treatment in patients with obesity in these age groups therefore require further study, in view of the observations now reported from which I conclude that fenfluramine interferes with pathways in the central nervous system regulating the release of HGH.

SUMMARY

1. In order to investigate the effect of fenfluramine on hormonal and metabolic changes with exercise, five normal volunteers have been studied during and after 20 minutes of steady exercise on a bicycle ergometer after injection of fenfluramine (20 mg i.v.).

2. Fenfluramine abolished the rise of plasma growth hormone which occurred in control investigations. Fenfluramine also affected plasma insulin, blood glucose and ketone-body levels.

3. The acute effect of fenfluramine on the release of growth hormone was examined further by studying its effect in patients with acromegaly. A marked depression of growth hormone occurred both at rest and with exercise.

4. These observations indicate that fenfluramine has a direct effect upon pathways controlling growth hormone release. It also suggests that this action may have practical use in the medical treatment of acromegaly.

CHAPTER 11

ORAL GLUCOSE LOAD AFTER NICOTINIC ACID

INTRODUCTION

In chapter 8 it was shown that oral administration of fenfluramine results in a significant improvement in glucose tolerance after the ingestion of glucose by patients with obesity. It was suggested that a part of its action may be due to a direct effect on factors regulating the peripheral uptake of glucose. Increased rate of fat utilization is known to upset the peripheral metabolism of glucose as in obesity and diabetes (Randle et al., 1963). Drugs that lower fat mobilization such as nicotinic acid may provide additional information about the effect of drugs on carbohydrate metabolism.

The administration of nicotinic acid causes depression of blood cholesterol, FFA and ketone bodies (Gurian and Adlersberg, 1959; Carlson and Oro", 1962; Bjorntorp", 1965). These changes may be secondary to the inhibition of fat mobilization by nicotinic acid.

Factors such as effects of rising blood glucose and insulin might also influence the concentrations of fat metabolites.

The reports on the effect of nicotinic acid on blood glucose are contradictory. Both hyper and hypoglycemia have been reported (Mirsky et al., 1957; Parsons, 1961), although an impairment of carbohydrate tolerance seems to be more generally accepted in diabetes mellitus particularly (Molnar et al., 1964). Little is known about the effect of nicotinic acid on insulin metabolism. It has been shown that the drug has an insulinase inhibitory effect in man (Mirsky et al., 1957). To examine the effects of nicotinic acid on carbohydrate metabolism a glucose

tolerance test was carried out and plasma IRI and HGH were determined during the test.

METHODS

Subjects: Seven subjects, four female (aged 30, 40, 50, 52 yr) and three male (aged 54, 58, 62 yr) were studied on three mornings. They had mean heights of 163 cm and mean weights of 63 kg. The subjects were volunteers, none of whom was known to have abnormal fat and carbohydrate metabolism.

Procedures:

1. Control glucose tolerance test. After catheterization, the subjects ingested 50 g glucose orally within 2 min. Blood samples were taken beforehand and at 15 min intervals for the first hour. Further blood samples were also taken at 30 min intervals for a further 2 h.

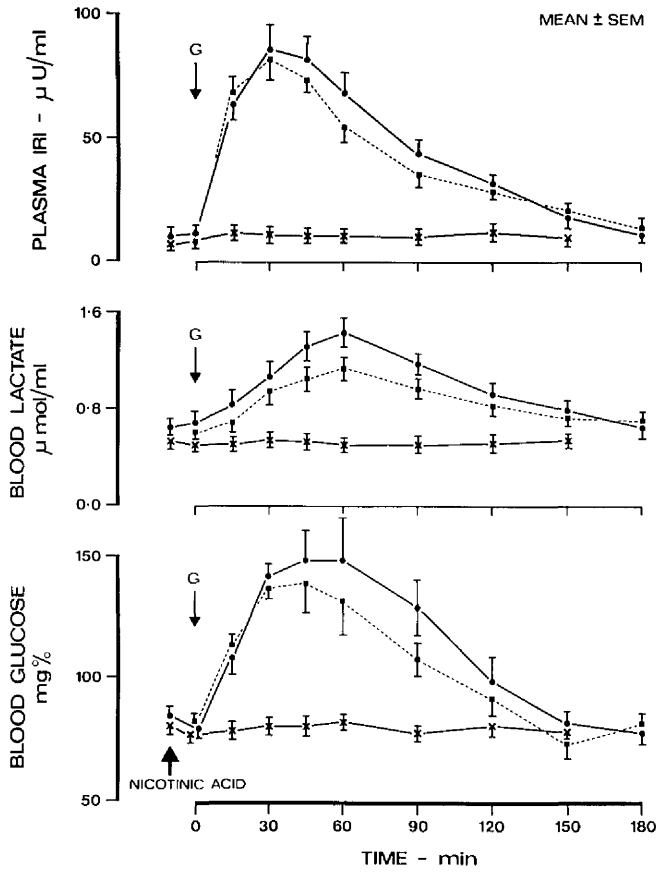
2. Glucose tolerance after nicotinic acid. On second occasion, a glucose tolerance test was performed as described above except that nicotinic acid (20 mg) was given orally 10 min before the glucose load.

Control study with nicotinic acid alone. On the third occasion nicotinic acid (20 mg) was given alone and blood samples were taken for 150 min in the same manner as on the previous occasions.

RESULTS

Glucose (fig 11.1): Nicotinic acid given alone had no effect on the fasting concentration of blood glucose, but it caused a decrease

Fig (11.1): The changes (mean ± SEM) in seven subjects of blood glucose (mg%), plasma insulin (μU/ml) and blood lactate (μ mol/ml) after a control glucose load (■- - - - - - ■), glucose load after nicotinic acid administration (●———●), and after nicotinic alone (╳———╳).


MEAN ± SEM
PLASMA IRI - μ U/ml
100
50
0
G
BLOOD LACTATE
μ mol/ml
1·6
0·8
0·0
G
BLOOD GLUCOSE
mg %
150
100
50
G
NICOTINIC ACID
0
30
60
90
120
150
180
TIME - min

of glucose tolerance when it was given before the glucose load. After nicotinic acid the concentrations of glucose were higher, but significantly different from the control values at only 90 min ($P < 0.05$).

Plasma IRI (Fig 11.1): Fasting concentrations of IRI were similar on the three occasions. Nicotinic acid alone did not alter them. The glucose load caused a rise in the concentration of IRI to a peak at 45 min on both with or without nicotinic acid. With nicotinic acid the concentrations were relatively higher, but not significantly different from that of the control at any point throughout the test. The concentrations then returned to resting values by the end of the investigation.

Lactate (Fig 11.1): Fasting concentrations of blood lactate were similar on the three occasions. They rose after glucose load both in the control and after nicotinic acid to a maximum at 60 min, but with nicotinic acid alone the concentrations remained unchanged. During the glucose tolerance test lactate concentrations were relatively higher with nicotinic acid compared with those of the control investigation, but were significantly different at 45, 60 and 90 min after glucose ingestion ($p < 0.05$).

DISCUSSION

In this study of glucose tolerance after the oral administration of nicotinic acid the concentrations of glucose were higher after nicotinic acid than control values although significance was only reached at 90 min. This intolerance to glucose, although of only a small degree, occurred despite similar response of insulin in both studies of

glucose tolerance. The lactate concentrations were significantly higher during the test after nicotinic acid. Uptake of glucose by muscle in the presence of insulin is impaired in diabetes, acromegaly and after dietary restriction (Himsworth, 1939; Butterfield, 1961). In those disorders HGH concentrations are increased and plasma FFA is frequently raised. FFA has been shown to reduce sensitivity to insulin by impairing phosphorylation of glucose and diminishing the effect of insulin on membrane transport of glucose (Kipnis, 1959; Newsholme and Randle, 1961; Randle et al., 1963). Impaired glucose tolerance after nicotinic acid administration is, however, unlikely to be due to increased tissue resistance to insulin via changing FFA and HGH, as nicotinic acid and glucose caused a fall in plasma FFA and HGH rather than a rise.

Impairment of glucose tolerance might also be an effect of nicotinamide, a metabolite of nicotinic acid. Nicotinic acid does not directly interfere with tissue respiration, but nicotinamide produces hyperglycemia (Bergman and Wislicki, 1953). This occurs as a result of suppression of tissue respiration, with inhibition of the activity of those enzymes which contain nicotinamide such as NAD and NADP (Fiegelson et al., 1951).

Since nicotinic acid had no effect on fasting concentration of blood glucose when it was given alone and since it is known to cause liver damage (Parsons, 1961), it is possible that its effect may depend upon a delay in glucose uptake by hepatic tissues. In the liver, the rate limiting step for glucose uptake is controlled by hexokinase. It is possible that nicotinic acid may facilitate glycogen breakdown by lowering plasma FFA resulting in increased production of glucose-6-phosphate.

This would inhibit the activity of hexokinase and thus hepatic uptake of glucose. Metabolism of glucose would then proceed and be the explanation of the increase in lactate concentration. Whether the effect of nicotinic acid on glucose tolerance via nicotinamide or secondary to the lowered plasma FFA is unclear. The effect of nicotinic acid on fat mobilization and related effects have therefore been studied during exercise and the results are given in the next chapter.

SUMMARY

1. To investigate the effect of nicotinic acid on carbohydrate metabolism, seven healthy subjects were studied after oral glucose with and without the drug. The subjects were also studied when nicotinic acid was administered alone. Venous blood samples were taken at regular intervals and were analysed for glucose, lactate, FFA, IRI and HGH.

2. The hyperglycemia and lactaemia after the administration of glucose was increased with nicotinic acid. The rise in plasma IRI in response to the glucose load was similar with and without nicotinic acid. The effects of nicotinic acid on blood glucose and lactate concentrations were not produced when the drug was given alone.

3. These studies suggest that nicotinic acid caused the disposal of glucose to be reduced and anaerobic glycolysis to be increased, even though insulin release was not altered by the drug.

CHAPTER 12

EXERCISE AFTER NICOTINIC ACID

INTRODUCTION

The studies of oral administration of nicotinic acid reported in the previous chapter showed a significant increase in blood lactate concentration after glucose ingestion compared with control studies. A minor degree of decreased tolerance was also observed. Whether these effects of nicotinic acid result from interference with tissue respiration or were secondary to the changes in FFA is not clear. Studies have been conflicting during exercise. Carlson et al., (1963), found that during exercise nicotinic acid had no significant effect on the concentration of blood lactate. Bergström and his colleagues (1969), however, found a significant increase in blood lactate associated with a decrease in glycogen in working muscle. Since most of the metabolic processes during exercise are under the control of endocrine activity, it is possible that the drug may alter the pattern of hormonal activity in response to exercise. It was the purpose of the present study to examine the effect of nicotinic acid on the pattern of metabolic and hormonal responses to exercise.

METHODS

Subjects: Five healthy male volunteers (aged 24 - 39 yr; mean height 174 cm; mean weight 78 kg).

Procedure: The subjects exercised on a bicycle ergometer at 600 kpm for 30 min on two separate occasions after fasting overnight. Blood samples were taken from a venous catheter. On the second occasion nicotinic acid (20 mg) was given orally between resting samples and

exercise was started 10 min later. The techniques for catheterization, handling of blood samples and monitoring the heart rate are described in appendices I and II.

RESULTS

Nicotinic acid administration caused a generalized flush and a feeling of warmth about 10 min after the beginning of exercise. It gradually disappeared about 50 - 60 min after taking the tablets. One of the subjects developed a skin rash around the elbows and knees.

Heart rate: The changes in heart rates during exercise and recovery were similar on both occasions.

Blood metabolites

Pyruvate (Fig 12.1): Resting pyruvate concentrations were not significantly different. They rose to a maximum at 15 min of exercise in the control study whereas with nicotinic acid the maximum occurred at 20 min of exercise. Pyruvate concentrations were higher with nicotinic acid both during exercise and in the recovery period, but significance was reached at only 20 min during exercise ($p < 0.05$).

Lactate (Fig 12.1): At rest blood lactate concentrations were similar and rapid elevation occurred during exercise. The concentration was significantly higher after nicotinic acid ($p < 0.05$) at 25 min during exercise and at 15 min afterwards.

FFA and Glycerol (Fig 12.2): At rest the concentrations of plasma FFA were significantly lowered by nicotinic acid ($p < 0.05$).

Fig (12.1): Blood pyruvate and lactate levels (μ mol/ml; mean ± SEM) in five subjects before, during and after two periods of 30 min exercise.
Control period (■------■); with nicotinic acid (●——●).

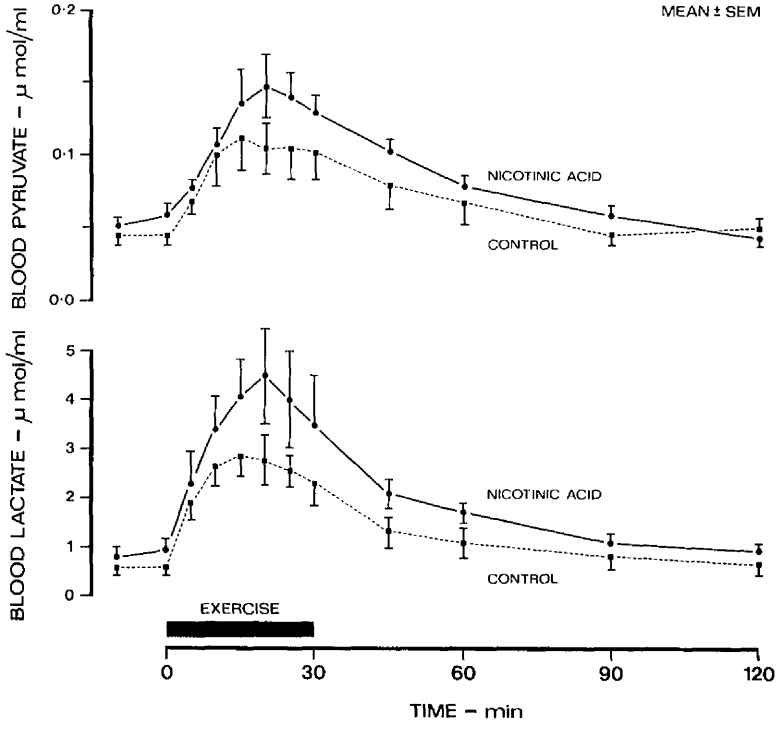
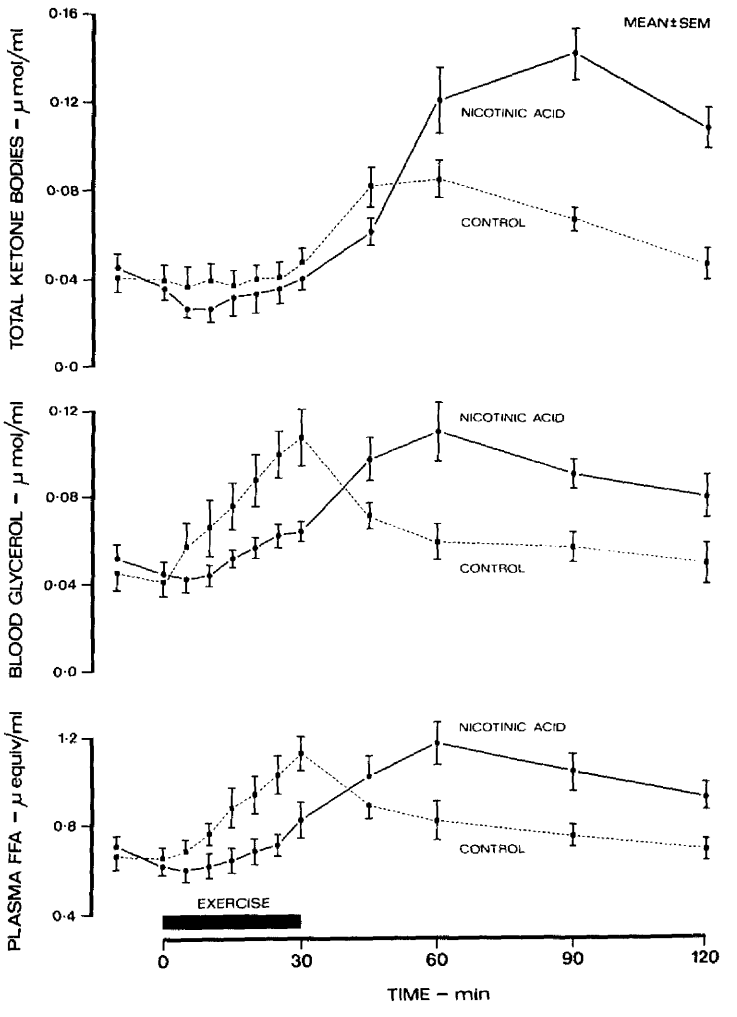

MEAN ± SEM
BLOOD PYRUVATE – μ mol/ml
0·2
0·1
0·0
NICOTINIC ACID
CONTROL
BLOOD LACTATE – μ mol/ml
5
4
3
2
1
0
NICOTINIC ACID
CONTROL
EXERCISE
0
30
60
90
120
TIME – min

Fig (12.2): Plasma FFA (μ equiv/ml), blood glycerol and ketone bodies (μ mol/ml; mean ± SEM) in five subjects before, during and after two periods of 30 min exercise. Control period (■- - - - - ■); with nicotinic acid (●————●).


MEAN±SEM
TOTAL KETONE BODIES – μ mol/ml
0·16
0·12
0·08
0·04
0·0
NICOTINIC ACID
CONTROL
BLOOD GLYCEROL – μ mol/ml
0·12
0·08
0·04
0·0
NICOTINIC ACID
CONTROL
PLASMA FFA – μ equiv/ml
1·2
0·8
0·4
NICOTINIC ACID
CONTROL
EXERCISE
0
30
60
90
120
TIME – min

During exercise the concentrations rose in the control study to a maximum at the end of exercise. With nicotinic acid, however, the rise was abolished ($p < 0.001$). In the control investigation the concentration returned to the resting value 60 min after exercise, whereas after nicotinic acid the concentrations rose only after the end of exercise to a maximum 60 min later. The concentration then returned slowly but remained above the resting value. The changes in glycerol concentrations on the two occasions were similar in pattern to those of plasma FFA.

Ketone-bodies (Fig 12.2): Resting levels of total ketone-bodies were similar and concentrations at the end of 30 min of exercise were not significantly different. After exercise the concentrations increased considerably both in the study after ingestion of nicotinic acid and the control investigation. The maximum values occurred in the study with nicotinic acid and they were observed 30 min after the maximum in the control study. The peak values were significantly different ($p < 0.01$).

Glucose and plasma IRI (FIG 12.3): The concentrations of blood glucose and plasma IRI were not significantly different at any time throughout the investigation, although they fell during exercise and rose immediately afterwards.

Plasma HGH (Fig 12.4): Resting plasma HGH concentrations were not significantly different on the two occasions. The concentrations rose to a maximum at the end of exercise, where the concentration was significantly higher after nicotinic acid administration ($p < 0.05$). In the control investigation the concentrations returned to resting values by the end of the investigation, whereas with nicotinic acid the concentrations

Fig (12.3): Blood glucose (mg%) and plasma IRI (μU/ml; mean ± SEM) in five subjects before, during and after two periods of 30 min exercise. Control period (■- - - - - ■); with nicotinic acid (●———●).

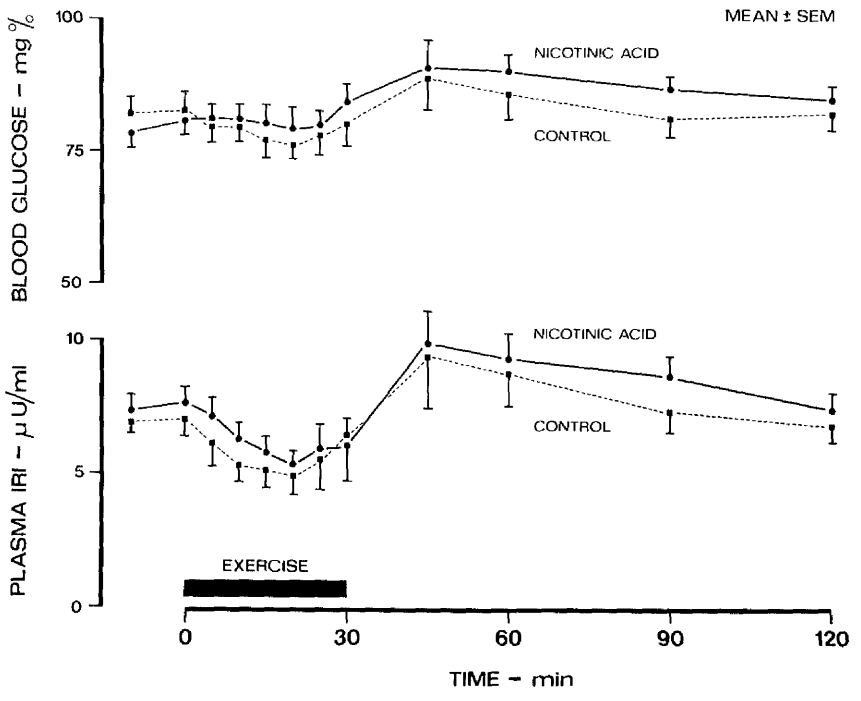
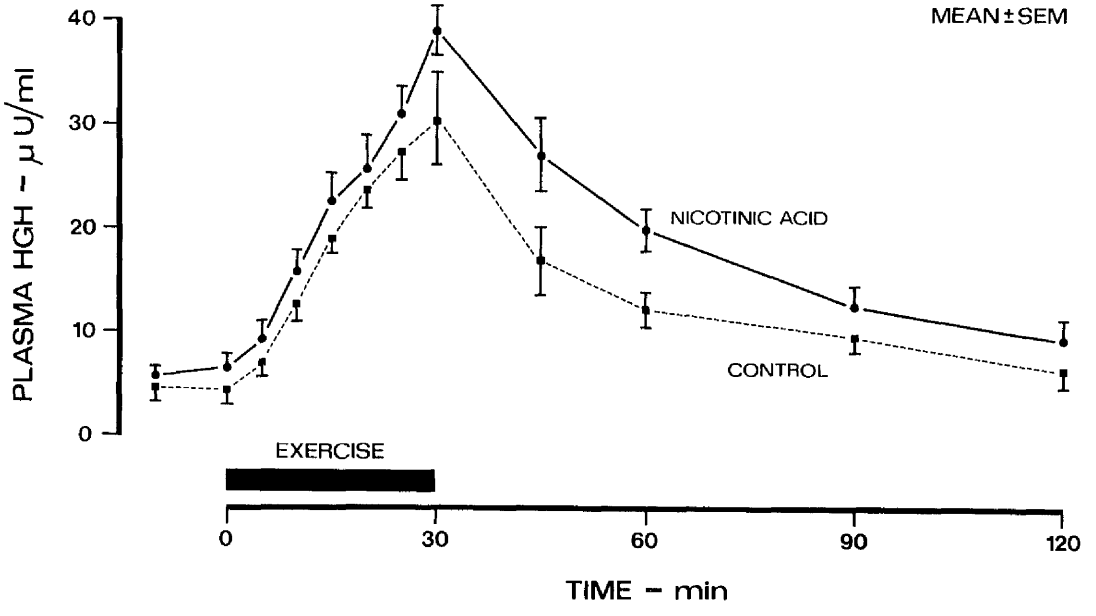

MEAN ± SEM
BLOOD GLUCOSE – mg %
100
75
50
NICOTINIC ACID
CONTROL
PLASMA IRI – μU/ml
10
5
0
NICOTINIC ACID
CONTROL
EXERCISE
0
30
60
90
120
TIME – min

Fig (12.4): Plasma HGH (μU/ml; mean ± SEM) in five subjects before, during and after two periods of 30 min exercise. Control period (■- - - - - - ■); with nicotinic acid (●————●).


MEAN±SEM
PLASMA HGH - μU/ml
40
30
20
10
0
NICOTINIC ACID
CONTROL
EXERCISE
0
30
60
90
120
TIME - min

of HGH remained higher in the post-exercise period, and were significantly different at 15 min after exercise ($p < 0.05$).

DISCUSSION

Nicotinic acid reduced the rise of FFA and glycerol which normally occur during exercise. Increased fat mobilization during exercise probably results from increased adrenergic activity (Basu et al., 1960; Havel et al., 1963). Nicotinic acid prevents the increase in the concentration of FFA which follows injection of nor-adrenaline (Carlson and Oro, 1962) and this action may account for the failure of FFA concentrations to rise during exercise after the administration of nicotinic acid.

At the end of exercise both FFA and glycerol concentrations rose dramatically with nicotinic acid to a maximum 30 min after exercise. Similar findings at rest have been reported by Pereira (1967) and he suggested they might be due to greater activity of the pituitary and adrenal glands. The greater response of HGH during exercise in the study with nicotinic acid compared with the control occasion is in keeping with this suggestion. The mechanism by which nicotinic acid stimulates the release of HGH is not clear, but since FFA supply was diminished after nicotinic acid, it is possible that HGH release was exaggerated as a feedback response (Tshushima et al., 1970).

There was probably no difference between production and utilization of ketone-bodies on both occasions, as the concentrations hardly alter during exercise. Normally in the post-exercise period when

the utilization of ketone-bodies as a fuel is decreased, there is a rise in ketone-body concentrations (Courtice and Douglas, 1936; Johnson et al., 1969). As with FFA, the increase was greater after nicotinic acid suggesting that the drug had not blocked FFA oxidation in the liver.

The higher lactate and pyruvate concentration during exercise after the administration of nicotinic acid suggests that the drug either increased the rate of glycolysis or decreased the rate of lactate disposal. The latter possibility seems unlikely, however, since the ratio of lactate/pyruvate and the rate of their disappearance was similar on both occasions. In addition, Bergström et al., (1969) have found that after the administration of nicotinic acid much glycogen is decreased during exercise. It seems more likely, therefore, that the rate of lactate production was increased consequent on greater glycogen breakdown. Lactaemia may have been exasperated by lactate entering the circulation from the liver as a result of sympathetic activation of hepatic glycolysis.

In studies reported in the previous chapter glucose tolerance was impaired after nicotinic acid administration and lactate production was increased. It was suggested that this action of the drug may depend upon a delay of hepatic uptake of glucose and not be due to a defect in the peripheral metabolism of glucose.

In the present study the blood glucose concentration did not rise with nicotinic acid after 30 min of exercise implying that there was an increase in the rate of glucose utilization possibly due to the acute reduction of plasma FFA enhancing the utilization of glucose by active muscle (Bergström et al., 1969; Lassers et al., 1972).

SUMMARY

1. To investigate the effects of nicotinic acid on metabolic and hormonal changes with exercise, five healthy volunteers have been studied during and after 30 min of moderate exercise on a bicycle ergometer after oral administration of nicotinic acid (20 mg).

2. Nicotinic acid caused a greater rise in pyruvate and lactate concentrations during exercise, compared with control investigation. Nicotinic acid also abolished the usual rise of both FFA and glycerol concentrations during exercise, but caused a massive release of FFA and glycerol in the post exercise period. After exercise the rise of ketone bodies concentrations was greater after nicotinic acid administration. HGH response to exercise was also altered by nicotinic acid.

3. These observations indicate that nicotinic acid interferes with the mobilization of fuel substrate during exercise, thus inhibiting fat mobilization and enhancing the rate of glycolysis.

CHAPTER 13

ETHYL ALCOHOL AND HUMAN GROWTH HORMONE RELEASE

INTRODUCTION

Studies on the release of growth hormone in normal subjects and in patients with acromegaly or with diabetes mellitus have been described in chapter 9 and 10. Although the mechanisms involved in the secretion of growth hormone from the pituitary gland have not been delineated, experimental observations suggest that the hypothalamus plays an important role in this process by releasing an intermediary neurohormone (Martin, 1973). It was therefore, thoughtlikely that alteration of growth hormone secretion would occur with drugs affecting hypothalamic activity. Evidence supporting this was provided by the observations that fenfluramine, an appetite depressant agent which is known to deplete amines in the hypothalamus (Costa et al., 1971; Bianc et al., 1972) reduced the rise of growth hormone induced by exercise in normal subjects and also in patients with acromegaly. It was suggested in chapter 10 that fenfluramine has a direct effect on pathways in the hypothalamus regulating the release of growth hormone.

Ethanol is also known to interfere with the metabolism of cerebral amines (Cursey and Olson, 1960). It is possible that alcohol may also affect the release of growth hormone. Although a rise in plasma HGH and cortisol after alcohol administration has been recognised (Bellet et al., 1970; Bellet et al., 1971), no information appears to be available about the possible effect of alcohol on plasma growth hormone concentration during exercise. Studies were therefore designed to examine the effect of acutely administered alcohol on growth hormone release during subsequent exercise in normal subjects. Since hypothalamic

and pituitary function is known to be disturbed in patients with chronic alcoholism (Merry and Marks, 1969), exercise studies in this situation may provide additional information about the role of the hypothalamus in regulating the release of growth hormone. The exercise response of six subjects with chronic alcoholism was also studied.

These studies were carried out in association with Mr. R.J. Chalmers who was interested in the metabolic response to exercise after alcohol administration. Assays of growth hormone and insulin during these investigations however, were personally made and these results constitute the subject of this chapter.

METHODS

Effect of ethyl alcohol on growth hormone release in normal volunteers:

Subjects: Six trained male athletes (age 20 - 23 yr, mean ht 174 cm; mean wt 68 kg) participated in this investigation. The subjects were students at Jordanhill College for physical education. None of them had taken alcohol on the day before the investigation and they were taking normal unrestricted diet.

Procedure: The subjects exercised by running round a track in the gymnasium on two separate occasions. On both occasions the subjects had fasted overnight and exercised for 30 min covering distances from 4 - 6 km. Blood samples were taken from a catheter placed in an antecubital vein. Two samples were taken at rest; further samples were taken at 10 min intervals during exercise and at 5, 20, 50 and 80 min after

exercise. The exercise was interrupted on the completion of each 10 min of exercise period for blood sampling. On the second occasion they drank 0.5 g/kg body weight of ethyl alcohol between the resting samples and started the exercise 10 min later.

RESULTS

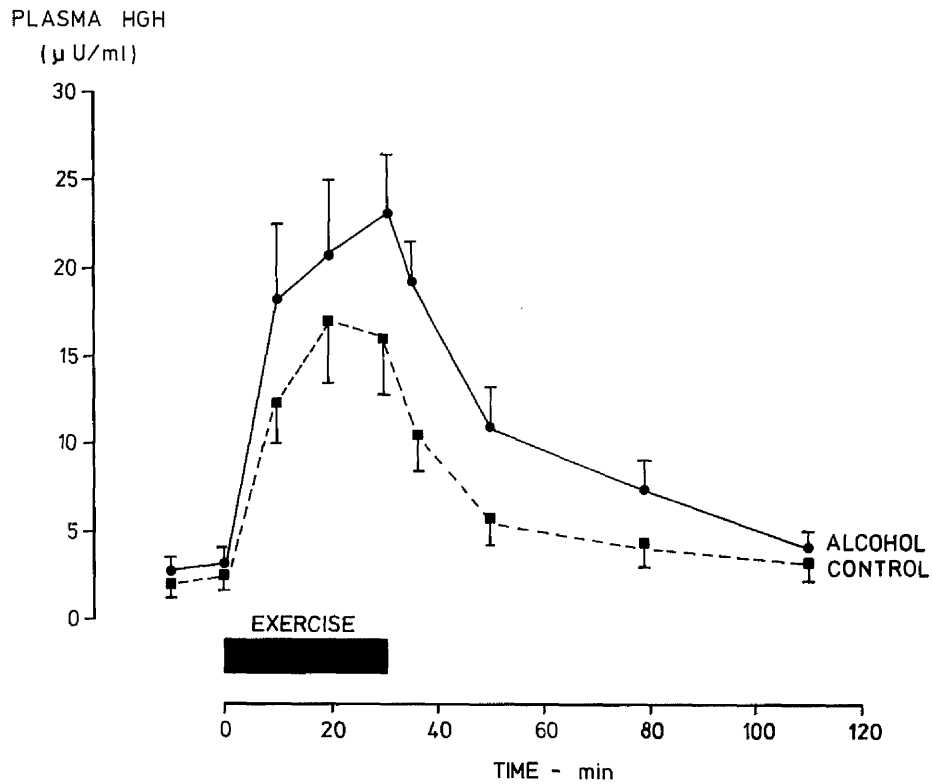
Plasma HGH (Fig 13.1): Resting HGH concentrations were not significantly different with and without alcohol. They rose to a maximum at 20 min of exercise in the control study whereas with alcohol the maximum occurred at the end of exercise. Plasma HGH concentrations were higher with alcohol both during and after exercise but differences were significant only at the end of exercise and at 5 min after exercise.

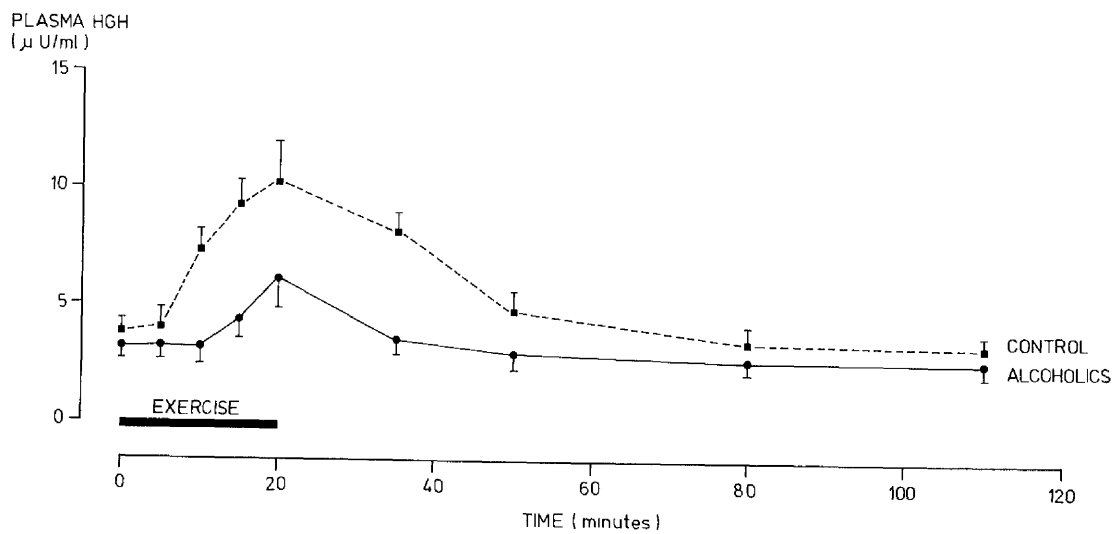
Lactate concentrations were similar at rest on both occasions. (Control study: 0.75 μmol/ml mean $\pm$ 0.3 μmol SEM; with alcohol: 0.72 μmol/ml mean $\pm$ 0.1 μmol SEM). During exercise the concentrations rose to a maximum at 10 min when there was no significant difference between the two occasions. (Control study: 8.4 μmol/ml mean $\pm$ 1.3 μmol SEM; with alcohol: 8.1 μmol/ml mean $\pm$ 0.7 μmol SEM). Lactate concentrations fell during both the remaining part of exercise and the recovery period in both investigations, but with alcohol, they were still significantly above the resting value 80 min after the end of exercise (2.6 μmol/ml mean $\pm$ 0.4 μmol SEM; $p < 0.01$).

The concentrations of blood pyruvate increased rapidly during exercise to achieve maximum at 10 min of exercise on both studies. The maximum values were significantly lower with alcohol (0.19 μmol/ml mean $\pm$

Fig (13.1): Plasma HGH (μU/ml; mean ± SEM) in six healthy subjects during and after two periods of 30 min exercise. control period (■- - - - - - ■); with alcohol (●————●

Fig (13.2): Plasma HGH (μU/ml; mean ± SEM) in five healthy control subjects (■- - - - - - ■) and six patients with chronic alcoholism (●————●) during and after 20 min of exercise.


PLASMA HGH
(μ U/ml)
30
25
20
15
10
5
0
ALCOHOL
CONTROL
EXERCISE
0
20
40
60
80
100
120
TIME - min


PLASMA HGH
(μ U/ml)
15
10
5
0
CONTROL
ALCOHOLICS
EXERCISE
0
20
40
60
80
100
120
TIME (minutes)

0.03 μmol SEM) than the control investigation (0.23 μmol/ml mean ± 0.05 μmol SEM). The pyruvate concentrations also remained significantly lower both during the remaining period of exercise and for 30 min afterwards ($p < 0.05$) with alcohol compared with control study.

The lactate/pyruvate ratio increased during exercise but recovered very quickly after exercise in the control observations. Alcohol however led to higher lactate/pyruvate ratios during exercise which remained elevated.

Blood glucose changed very little during exercise on the control investigation. With alcohol however, blood glucose had fallen by 25% by the end of exercise and fell a further 20% on the post-exercise period.

The changes in plasma FFA and IRI concentrations showed no differences between the control study and that with alcohol.

Effect of exercise on growth hormone release on patients with chronic alcoholism

Subjects: Six male subjects (age 40-48 yr; mean ht 173 cm; mean wt 68 kg) with chronic alcoholism and five healthy male controls (age 26-40 yr; mean ht 176 cm; mean wt 70 kg) were studied. The patients were studied 1 - 2 weeks after they had been admitted to the psychiatric unit for treatment of their chronic alcoholism. None of them had taken alcohol since admission.

Procedure: Patients and controls attended to the laboratory after an overnight fast. The nature and the effects of the investigation had been explained to the patients who had all volunteered to participate

in it. Subjects exercised for a period of 20 min on a bicycle ergometer at a fixed work load of 600 kpm/min. Heart rate was recorded during exercise and for further 15 min afterwards using an electrocardiograph. A catheter was placed in the arm vein and blood samples were taken before exercise, at 5 min intervals during exercise and then at 15, 30, 60 and 90 min after exercise.

In all investigations the blood samples were treated as described in appendices I and II and personally analysed for HGH, IRI and for metabolites by Mr. Chalmers.

RESULTS

The changes in lactate, glucose, FFA and IRI concentrations showed no differences between the controls and the alcoholics.

Plasma HGH (Fig 13.2): Plasma HGH concentrations were similar at rest in the patients and the controls. HGH concentrations rose in both groups. The highest value was achieved at the end of exercise, but the concentrations were 40% higher in the controls (10 μU/ml mean $\pm$ 1.5 μU SEM) than in the patients (6 μU/ml mean $\pm$ 0.8 μU SEM). In patients and controls HGH concentrations fell in the period following exercise to approximately pre-exercise values at 60 min after the end of exercise.

DISCUSSION

These results indicate that the release of growth hormone with exercise is greater following the ingestion of alcohol by normal subjects. The means by which alcohol produces such a change during exercise are

still unknown. Hypoglycemia (Hunter et al., 1965), fall of plasma FFA (Hartog et al., 1965) and a rise of blood lactate (Sutton et al., 1969) have been reported as a possible stimuli for producing an increase in plasma HGH concentration during exercise. The last two possibilities appear to be unlikely in this situation, because the changes of plasma FFA and blood lactate during exercise were similar with and without alcohol. The findings of a significant drop in blood glucose and a rise in plasma HGH concentrations during exercise with alcohol suggest the possibility that hypoglycemia might be a factor. There is also the possibility that alcohol produced the greater elevation of HGH by sensitizing the release mechanisms to such other stimuli.

The secretion of HGH from the pituitary gland is controlled by the hypothalamus through an intermediary neurohormone system which is probably dependent on catecholamines transmission (Forhman, 1972; Martin, 1973; Merimee and Rabin, 1973). Gursey and Olson (1960) have shown that in the rabbit the administration of ethanol is followed by a rapid release of serotonin and nor-adrenaline from brainstores. It is therefore possible that alcohol interferes with HGH production by directly affecting the aminergic activity of the hypothalamus. The reports of Jenkins and Connolly (1968) and other workers (Bellet et al., 1971; Merry and Marks, 1972) who found that in normal individuals an amount of alcohol sufficient to produce mild to moderate intoxication, stimulates pituitary and adrenal activity as assessed by the release of cortisol and HGH. This and the present observation of a greater growth hormone response to exercise after alcohol by normal subjects are consistent with an acute

stimulating effect of alcohol on pathways regulating the release of HGH.

Merry and Marks (1969) observed that plasma cortisol concentrations in patients with chronic alcoholism were paradoxically depressed after alcohol administration. They suggested that chronically self administered alcohol has a depressor effect on hypothalamic pituitary function. This suggestion is supported by the present observation of a lower growth hormone response to exercise in chronic alcoholics.

SUMMARY

1. In order to investigate the effect of alcohol on hormonal and metabolic changes with exercise, six patients with chronic alcoholism were studied. Six healthy volunteers were also studied during and after 30 min of exercise following ingestion of alcohol (0.5 g/kg body weight).

2. Acutely administered alcohol caused an increased response of growth hormone. Alcohol also affected blood glucose, pyruvate and lactate.

3. In chronic alcoholism the growth hormone response to exercise was reduced.

4. These observations indicate that alcohol acts acutely by sensitizing the release mechanism for growth hormone probably in the hypothalamus.

5. The failure to obtain normal increase in plasma growth hormone during exercise in patients with chronic alcoholism suggests that chronic abuse of alcohol suppresses or reduces the ability of the central nervous system to release growth hormone.

CHAPTER 14

GENERAL SUMMARY AND CONCLUSIONS

In the introduction to this thesis I explained that the purpose of these investigations was first to establish the usual response of blood metabolites and hormones to various stimuli and in particular exercise. The second purpose was to compare the responses in altered nutritional or pathological states and following the administration of some pharmacological agents such as fenfluramine, nicotinic acid and alcohol. Exercise was chosen because it produces greater demands on the metabolic processes. In this situation any metabolic or hormonal effect of pharmacological agent, pathological or nutritional states could be expected to be magnified.

The finding reported in chapters 1, 2, 3 and 6 demonstrated that the size and the direction of blood metabolites and hormones changes during exercise vary with physical fitness, nutritional state and pathological conditions.

Compared to healthy untrained subjects, trained athletes efficiently mobilize fuels stored as glycogen in liver and fat in adipose tissue as indicated by the amount of glucose and glycerol released. In addition they have lower concentrations of blood lactate and pyruvate during exercise and of plasma FFA and blood ketone-bodies during and after exercise. This would indicate that the FFA mobilized during exercise is apparently more efficiently oxidized in trained athletes. In untrained subjects however, incomplete oxidation of fat causes a considerable rise in blood ketone-bodies after exercise. In addition to these biochemical adaptations, training appears to result in modifications in the endocrine activity. These are reflected in smaller responses of growth hormone and insulin in physically trained individuals than untrained subjects during exercise.

Plasma insulin levels are known to be decreased both by lower blood glucose concentrations and by increased catecholamine concentrations (Ellis, 1962; Mayhew et al., 1969). The changes of blood glucose is not in the same direction as plasma insulin (chapter 1), and the reason for this is not clear but the increasing levels of catecholamines in response to exercise may reduce the insulin response. Lower blood catecholamines have been reported in man after a training programme (Hartley et al., 1972) and this may permit the higher concentrations of insulin in athletes.

The studies on the metabolic effects of obesity and therapeutic starvation described in chapter 2 and 3 demonstrated that energy is provided apparently more from the oxidation of fat and fat derived substrates. This was more marked during starvation as indicated from the size and the direction of both FFA and ketone-bodies changes. In starvation utilization of ketone-bodies occurred during exercise in spite of the availability of glucose. This is in keeping with the suggestion of Owen and co-workers (1967) that in starvation, the utilization of ketone-bodies could be an adaptation by the body to spare glucose for the requirements of the nervous system. In addition to these adaptations, starvation also sensitizes the mechanism for growth hormone release. Growth hormone is a potent lipolytic hormone which may act during starvation to mobilize more fat for energy demand.

The studies on the relationship of ketone-bodies to insulin release described in chapter 4 demonstrated that in man the administration of ketones stimulates the release of insulin which in turn caused the fall of plasma FFA and blood glucose. These observations support the suggestion

of Mebane and Madison (1964) that hyperketonemia by stimulating insulin modulates fat mobilization from adipose tissue and limits the production of ketone body. Evidence for this suggestion was provided by the high correlation ($r = .97$: $P < 0.05$) between the highest observed concentrations of plasma insulin and blood ketone-bodies in patients with acromegaly, who showed an abnormal peak in blood ketone-bodies and plasma insulin during exercise, but hardly any post-exercise ketosis (chapter 5).

The studies on the patterns of metabolic and hormonal changes during exercise in normal man and in patients with obesity (chapter 1, 2 and 3) provided a background from which to compare fat and carbohydrate metabolism in patients with diseases of muscle. One disorder studied here was a case of oculoskeletal myopathy. In this patient, histochemistry and electron microscopy demonstrated an ultrastructural abnormality of the muscle mitochondria. There were also metabolic abnormalities with exercise. Blood lactate and pyruvate rose, but to a much greater extent than normally expected. Concentrations of ketone-bodies and FFA fell during and after exercise whilst they rose in the controls.

Experience gained from the previous studies on the metabolic and hormonal responses to exercise in normal man and in patients with obesity or muscle disorders (chapter 1, 2, 3 and 6) have made it also possible to compare metabolism in man following the administration of pharmacological agents such as fenfluramine, nicotinic acid and alcohol.

Studies on the metabolic effect of fenfluramine in patients with obesity described in chapter 7 and 8 demonstrate that the action of fenfluramine may be due, at least in part, to a direct effect of the drug

on carbohydrate and fat metabolism. It improves glucose tolerance, possibly because glucose uptake by muscle is increased (Butterfield and Whichelow, 1968). Comparing the metabolic effects of this drug and the effect of dietary restriction, it was shown that concentrations of plasma FFA, blood glycerol and total ketone-bodies increased, but the size of these changes was much less with fenfluramine. The changes during fenfluramine therapy could have been due to appetite suppression since a reduction of dietary intake would facilitate fat mobilization and ketone body production. However an entirely metabolic effect of fenfluramine on ketone-body metabolism has been observed as reflected by the changes in the ratio of 3-hydroxybutyrate/acetoacetate which fell after the administration of fenfluramine but rose with dietary restriction. These observations imply that the metabolic changes with fenfluramine include a direct effect, and are not necessarily secondary to reduced dietary intake.

Further observations were made of the effects of administration of fenfluramine intravenously in normal man (chapter 9 and 10). Studies in resting subjects reported in chapter 9 showed a decrease in plasma HGH concentrations. Since exercise stimulates growth hormone release, observations of the changes in growth hormone release during exercise were also made. Observations in animals have indicated that the hypothalamus is a possible site for the action of the drug (Anand, 1971; Anand and Blundell, 1971) and growth hormone release could be an index of hypothalamic activity (Frohman, 1972; Martin, 1973). Fenfluramine also abolished the rise of plasma HGH during exercise. This effect was examined further by studying patients with acromegaly (chapter 10), a condition in which plasma HGH is elevated. In these patients a marked depression of growth hormone occurred both at rest and during exercise. The secretion of growth hormone from the anterior pituitary gland is controlled by the hypothalamus through

an intermediary neurohormone system which is dependent on catecholamine transmission (Frohman, 1972; Martin, 1973). However, since fenfluramine depletes cerebral amines particularly in the hypothalamus (Costa et al., 1971; Ziance et al., 1972) an effect on catecholamine transmission may explain the failure of exercise to stimulate growth hormone production following fenfluramine injection.

The suppressive action of fenfluramine on growth release was studied further in patients with diabetes mellitus (chapter 9). In diabetes mellitus, plasma HGH concentrations fluctuate but are frequently found to be considerably elevated for long periods (Lundback, 1971). In the patients studied here, fenfluramine caused a fall in the concentrations of plasma HGH. Blood glucose concentrations were also reduced. This fall in blood glucose may be attributed to the increased peripheral uptake of glucose (Butterfield and Whichelow, 1968). From these observations it was concluded that fenfluramine interferes with central nervous system regulation of the release of growth hormone, and also has a direct metabolic effect on processes in peripheral tissue regulating fat and carbohydrate metabolism.

Studies on the metabolic effect of nicotinic acid described in chapter 11 and 12 demonstrate that this drug interferes with one or more of the metabolic pathways regulating fat and carbohydrate metabolism. It reduces glucose tolerance and enhances lactate production, possibly because glucose assimilation by the liver is reduced (Miettinen et al., 1969). Nicotinic acid also interferes with the mobilization of fuel substrates, inhibiting fat mobilization in adipose tissue and enhancing the rate of glycogenolysis in liver and muscle. The acute reduction of fat

mobilization following nicotinic acid administration appears to stimulate the release of growth hormone during exercise.

Studies on the metabolic effect of alcohol described in chapter 13 demonstrate that acutely administered alcohol causes an increased response of growth hormone to exercise in normal subjects. In patients with chronic alcoholism the response of growth hormone to exercise was reduced. These observations suggest that alcohol acts acutely by sensitizing the release mechanism for growth hormone probably in the hypothalamus. The failure to obtain a normal increase in plasma growth hormone during exercise in patients with chronic alcoholism suggests that chronic abuse of alcohol reduces the ability of the central nervous system to stimulate the release of growth hormone.

From these observations of the metabolic effects of pharmacological agents which affect fat and carbohydrate metabolism, I conclude that while they may act directly by an action on metabolic pathways in the liver or in peripheral tissues like muscle, they also act indirectly by modifying endocrine activity.

APPENDIX I

TECHNIQUES

Biochemical Techniques

A. Preparation of blood samples

14 ml blood was taken on each occasion from a forearm vein by an indwelling canula. 5 ml of this blood was immediately added to 5 ml 10% (V/V) ice-cold perchloric acid in a pre-weighed plastic universal container. This was shaken vigorously and stored in the cold until the protein precipitate could be centrifuged down. The remaining 10 ml of blood were placed in lithium heparinized tube and centrifuged for 10 min at 2,500 r.p.m. The separated plasma was pipetted off and stored at -10°C.

B. Treatment of protein free filtrate

After the removal of denatured blood by centrifugation at 18,000 r.p.m. for 20 min, the clear supernatant was decanted into a graduated tube and the volume noted. The specimens were neutralised with 20% potassium hydroxide (1.5-2 ml) using universal indicator. The tubes were re-centrifuged for 5 min and the volume noted again. The insoluble potassium perchlorate was allowed to precipitate completely by allowing the tubes to stand at 4°C for 30 min. The supernatant was then decanted into plastic tubes, which were stored at -10°C for subsequent analysis.

A dilution factor was calculated from the volume of blood taken and the volume of alkali added as follows :-

$$\frac{\text{Blood volume} + 5}{\text{Blood volume}} \times \frac{\text{Neutral volume}}{\text{Acid volume}}$$

Spectrophotometric measurements were carried out on Hilger & Watts UV spectrophotometer.

C. Analysis of blood pyruvate and blood acetoacetate

These two metabolites were measured on the same sample by modifications of the methods of Hohorst et al., (1959) for pyruvate and Williamson et al., (1962) for acetoacetate. The pyruvate was reduced to lactate with excess of NADH using a phosphate buffer (0.1M. pH 7.0).

$$\text{pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{pH 7.0}]{\text{LDH}} \text{L - Lactate} + \text{NAD}$$

The reaction was catalised by excess lactic dehydrogenase (Bohringer & Co., London) and the decrease in optical density due to the oxidation of NADH measured at 340nm. The acetoacetate was analysed under the same conditions by the enzyme D-(-)-β-hydroxybutyric dehydrogenase (Bohringer & Co., London) which catalyses the reaction of acetoacetate to 3-hydroxybutyrate.

$$\text{Acetoacetate} + \text{NADH} + H^+ \xrightarrow[\text{pH 7.0}]{\text{HBDH}} \text{3-hydroxybutyrate} + \text{NAD}$$

Acetoacetate is slowly decarboxylated to acetone in neutral solution, so this analysis had to be carried out immediately after the acid samples were neutralised.

D. Analysis of blood glucose

Blood glucose was determined by the method of Bergmeyer and Bernt (1963) using the enzymes glucose oxidase and peroxidase (Bohringer

& Co., London).

I. Glucose + O_2 + H_2O $\xrightarrow{\text{Glucose oxidase}}$ Gluconic acid + H_2O_2.

II. H_2O_2 + O-dianisidine $\xrightarrow{\text{peroxidase}}$ oxidised O-dianisidine.

III. Oxidised O-dianisidine + H^+ $\longrightarrow$ stable coloured solution.

Glucose oxidase catalyses the conversion of D-glucose to D-gluconic acid and the hydrogen peroxide formed is decomposed by peroxidase. The oxygen liberated oxidises O-dianisidine to form a stable orange brown complex at pH 7.0. The optical density of the reaction mixture was measured at 440 nm after incubation in the dark at 37°C for 1 hr.

Duplicate samples of 0.05 ml were analysed in relation to glucose standard of different concentration. 0.5 ml, 0.75 ml and 1.0 ml of 0.2 mM standard glucose was used.

E. Analysis of blood 3-hydroxybutyrate

This was determined by the method of Williamson et al., (1962) using the enzyme D-β-hydroxybutyric dehydrogenase to catalyse the oxidation of 3-hydroxybutyrate to acetoacetate in the presence of excess NAD.

$$\underset{\downarrow}{\underset{\text{Hydrazin}}{\text{Acetoacetate}}} + \text{NADH} + H^+ \underset{\text{pH 8.5}}{\overset{\text{HBDH}}{\rightleftharpoons}} \text{D-OH butyrate} + \text{NAD}$$

Acetoacetate was removed in the form of its hydrazone in alkaline medium by using hydrazine buffer (pH 8.5). The change in optical density due to the formation of NADH was measured at 340 nm.

F. Analysis of blood lactate

This was determined by the method of Hohorst et al., (1959) using the enzyme lactic acid dehydrogenase to catalyse the oxidation of lactate to pyruvate in the presence of excess NAD.

$$\text{L - lactate} + \text{NAD} \underset{\text{pH } 9.5}{\overset{\text{LDH}}{\rightleftharpoons}} \text{pyruvate} + \text{NADH} + \text{H}^+ \quad (\downarrow \text{Hydrazine})$$

An alkaline medium was used (pH 9.5) and the pyruvate removed in the form of its hydrazone.

The change in optical density due to the formation of NADH was measured at 340 nm.

G. Analysis of blood glycerol

This was determined by the method of Kreutz (1962) using the enzyme glycerokinase to catalyse the phosphorylation of glycerol by ATP to L-glycerol-1-phosphate. The ADP formed was phosphorylated back to ATP with PEP forming pyruvate which was then reduced to lactate with NADH in the presence of lactic acid dehydrogenase.

The whole reactions were as follows :-

$$\text{glycerol} + \text{ATP} \underset{\text{Mg}^{++}}{\overset{\text{G.K}}{\rightleftharpoons}} \alpha\text{-glycerophosphate} + \text{ADP}$$

$$\text{PEP} + \text{ADP} \overset{\text{P.K}}{\rightleftharpoons} \text{pyruvate} + \text{ATP}$$

$$\text{pyruvate} + \text{NADH} + \text{H}^+ \underset{\text{pH } 7.0}{\overset{\text{LDH}}{\rightleftharpoons}} \text{L-lactate} + \text{NAD}$$

Since 1 molecule of glycerol ≡ 1 molecule of NADH the optical density change at 340 nm due to the disappearance of NADH was proportional to the glycerol concentration.

H. Analysis of plasma FFA

Plasma FFA were determined by the method of Itaya and Ui (1965). Chloroform with pH 7.0 phosphate buffer of 0.1M was used to extract the plasma FFA. The extracted FFA were complexes with copper triethanol amine to soluble copper salt. After separation of the phases by centrifuging and removal of the top watery phase by filteration through ordinary filter paper, colorimetric estimation was carried out with the dye sodium diethyldithiocarbamate. The colour developed by the dye was measured immediately at 440 nm. All FFA determinations were carried out in duplicate using 0.2 ml samples of plasma in relation to palmitate standard of different concentrations. 1 ml, 2 ml, 3 ml, 4 ml and 5 ml of 0.1 mM palmitate standard was used.

APPENDIX II

RADIO-IMMUNO ASSAY

The development of radioimmunoassay techniques has made possible the routine measurement of physiological levels of many peptide hormones in biological fluids. The basis of the method is as follows :

The essential principle of these techniques is the competition for the binding site of a fixed amount of anti-hormone (anti-A) between a known amount of radioactively labelled pure hormone (A*) and unknown amount of unlabelled hormone (A). A+ anti-A + A* $\rightleftharpoons$ A: anti-A:A* + A*. As a result of the competition, the amount of labelled hormone bound to antibody will drop as the concentration of unlabelled hormone increases. After the reaction has been allowed to approach completion, the antibody-bound hormone is separated from free hormone and the distribution of radioactivity determined. The sensitivity of this reaction depends upon three requirements :

1. The availability of radio-iodinated hormone, whose affinity for antibody is similar to that of the unlabelled hormone.
2. An anti-serum having a high energy of reaction.
3. A double equilibrating incubation system (3 days at $4^{o}C$ for each).

To choose a sensible method of efficient separation, the following criteria should be considered :

1. Complete separation.
2. Simplicity, speed and reproducibility.
3. Ability to detect non-specific effects occurring during the assay, such as damage to labelled hormone and the effects of protein on both the antigen-antibody reaction and the separation procedure itself.

4. Cost of material.

5. Ease of processing a large number of samples.

The advantages and disadvantages of the most important techniques recorded in the literature may be assessed with these criteria :

A. Chromato-electrophoresis on Whatman paper: In this method the free hormone becomes absorbed on the paper at the point of application while antibody-bound hormone moves with plasma proteins. Chromato-electrophoresis as described by Yalow and Berson (1960) is a highly reliable method allowing excellent control of the reaction; elaborate apparatus is, however, required and the method is time-consuming.

B. Chemical precipitation: The antibody-bound hormone may be precipitated either with sodium sulphate (Grodsky and Forsham, 1960) or ethanol (Heding, 1966) under conditions in which the free hormone is left in the supernatant fluid. This method is simple and a large number of samples can be processed rapidly. The materials are relatively inexpensive. The disadvantage is that the non-specific effects of protein in this technique are considerable and difficult to control, making it unsatisfactory for plasma samples.

C. Differential adsorption onto solid material; charcoal: In this method the antibody-bound hormone remains in solution while free hormone is adsorped on charcoal. The adsorption of free hormone on charcoal described by Hunter (1971) is suitable for insulin. It is readily reproducible, fairly easy to perform and reasonably rapid. Furthermore charcoal allows detection of damage to labelled hormone and it is a relatively inexpensive material. Unlike insulin, adsorption of free human growth hormone (HGH) is dependent on the molarity and pH of the

buffer used in the system and, therefore, difficult to control.

D. Immunoprecipitation: As described by Morgon and Lazarow (1963) the antibody-bound hormone is precipitated by the reaction with a second anti-serum directed against the immunoglobulins of the anti-hormone serum. The method satisfies almost all the criteria except that the materials are relatively expensive. It also depends upon the availability of a second antibody specific enough to precipitate all the bound hormone. This method has been modified and used routinely as a standard method for HGH. It may also be used for insulin as a comparison with the charcoal technique.

Radio-immuno assay for Human Growth Hormone (HGH)

Further details of this two anti-body system are as follows :

1. Iodination of HGH: The method used is that of Greenwood et al., (1963) with minor modifications.

Materials

(a) (125-I) Iodide: Sodium (125-I) Iodide (IMS-3) (obtained from the Radiochemical Centre, Amersham). The material is specific between pH 7-9, free from reducing agents and carrier free the specific activity being 4-12 mC/μg Iodine.

(b) Human Growth Hormone: (obtained from the MRC biological Standard Division, London). Each vial contains approximately 1 mg of HGH (Raben preparation) with lactose. The material is dissolved in small amounts of bidistilled water, and if necessary a drop of 0.1N NaOH is added. The clear solution

is then diluted with 0.05M phosphate buffer to a concentration of 250 µg/ml and stored at -10°C.

(c) Chloramine-T: (B.D.H. Analar grade). A solution of 5 mg/ml in 0.05 buffer is freshly made up at the time of the iodination.

(d) Sodium metabisulphite (B.D.H. Analar grade): A solution of 5 mg/ml is freshly made up at the time of the iodination.

(e) Potassium iodide (B.D.H. Analar grade): A solution of 10 mg/ml is made up freshly on the day of the iodination and kept aside from other reagents.

(f) Bovine Serum Albumin: (B.D.H. Fraction V)

(i) 50 mg is dissolved in 1 ml of 0.07M barbitone buffer and placed in a polystyrene 5 ml vial for collection of the protein eluate from the column.

(ii) 20 mg is dissolved in 1 ml of the same buffer and is used to saturate the column of Sephadex G-50.

(g) Sephadex G-50: obtained from Pharmacia (Great Britain) Ltd. One gram of G-50 is added to 50 ml of 0.07M barbitone buffer the day before the iodination. The beads are allowed to settle in a beaker and the fines are poured as a thick slurry into a column 10CmX 1Cm, and washed with more buffer. The column is then saturated with bovine serum albumin by adding 1 ml of a 20 mg/ml solution directly on the top of the gel load. The protein is allowed to sink in and the column is then washed with at least 30 ml of 0.07M barbitone buffer.

(h) Buffers:

(i) pH 8.6 barbitone buffer 0.07 M. This is made up by dissolving 1.84 g of barbitone in 400 ml of hot distilled water followed by 10.3 g of sodium barbitone added to the cooled solution. The volume is then made up to 500 ml and stored at $4^{o}C$. Fresh dilutions of 3 parts of this concentrate buffer and 2 parts of distilled water are made up on the day of use.

(ii) pH 7.4 phosphate buffer 0.05M. This is made up by dissolving 5.68 g of Na_2HpO_4 and 1.33 g of NaH_2pO_4. H_2O in 100 ml of distilled water. This also can be stored at $4^{o}C$ and diluted 1 in 10 with distilled water.

Iodination procedure

The whole reaction is carried out in a fume cupboard. 10 µl of the isotope is drawn up using an oxford pipette and placed precisely at the bottom of the reaction vial (conical plastic tube clamped in a vertical position over a magnetic stirrer). Another 10 µl of isotope is added to a flask labelled "starting material" containing 200 ml of 1% KI in 0.07 M barbitone buffer. After addition of the isotope 20 µl of 0.5 M phosphate buffer pH 7.4 are added. The magnetic stirrer is then switched on and a small stirrer added (the stirrer consists of a small piece of steel wire sealed inside a glass capillary tube). From this point forward until the iodination reaction mixture is placed on the column

speed is essential. 5 µg of human growth hormone in 0.02 ml of 0.05M phosphate buffer is added. This is followed by 100 µg of chloramine-T in 0.02 ml of the same buffer. 30 seconds later, 500 µg of sodium metabisulphite in 0.1 ml of buffer is added to stop the reaction, followed by 2 mg of KI in 0.2 ml of buffer. The mixture is then transferred to the top of the sephadex G-50 Column. The reaction vial is washed with a further 0.4 ml of KI Solution and this is added to the Column once the initial mixture has sunk into the gel bed. The whole procedure takes about 5 min. The eluate fractions from the column are collected in 5 ml plastic vials using 0.07 M barbitone buffer pH 8.6.

Iodination flow Sheet

0.02 ml 0.5 M phosphate buffer

0.01 ml Isotope (125-I)

0.02 ml HGH in buffer = 5 µg

0.02 ml Chloramine-T

mixed for 30 seconds

0.1 ml Sodium metabisulphite

0.2 ml potassium iodide

Top of column Sephadex G-50

0.4 ml wash with potassium iodide

Fractions

1.5 ml Hold up volume (H.U.V.)

4.0 ml protein

5.0 ml salt peak (S1)

5.0 ml " " (S2)

5.0 ml " " (S3)

SETTING UP AN ASSAY

Reagents

1. 0.05M phosphate buffer pH 7.4-7.5.
This is made up by dissolving 2.85 g of Na_2HpO_4 and 0.67 g of KH_2pO_4 in 500 ml of hot distilled water. To this is added

Sodium EDTA	1.86 g
Bovine serum albumin	1.5 g
Sodium chloride	4.5 g
Sodium Azide	0.1 g

2. Human growth hormone standard. MRC standard A, calibrated against WHO international reference preparation is used for the assay. The stock standard of 250 µg/ml previously prepared for the iodination procedure is diluted with phosphate albumin buffer to 100 µU/ml, divided into 100 µl aliquots and stored at -20°C.

3. Rabbit anti-human growth hormone serum, DR16, Wellcome.
The content of each bottle (0.5 ml of 1:1000) is reconstituted with phosphate albumin buffer to a dilution of 1:8000.

4. Donkey anti-rabbit precipitating serum (DARS).
The content of each vial is diluted (1:5) with phosphate albumin buffer, divided into 5 ml aliquots and stored at -20°C.

5. Plasma samples for assay: the samples are thawed shortly before use and can be kept in a cool area at about 4°C until they have been used in the assay. All samples must be mixed well before use. They may

need to be centrifuged to remove strands of fibrin formed during storage that may otherwise interfere with the measurement of the volumes of samples to be used in the assay.

6. (^{125}I) Human growth hormone (see iodination page 139).

Assay procedure

The minimum number of samples in any one assay is 30. Each sample is assayed in duplicate and a standard curve is included in every assay (table 1). The standard consists of 14 tubes: two tubes as controls which contain antibody with labelled HGH, a further two as blanks which contain only labelled HGH, and the remaining tubes with increasing amounts of standard HGH (10 μU/ml). The final volume of each tube being made up to 1.0 ml diluent.

Table 1: Example of standard graph

Tube No.	Amount of HGH (μU/ml)	Diluent (ml)	% Bound	Graph reading	Anti-Log	Undiluted HGH μU/ml
1A	—	1.0	64			
2A	0.25	0.975	51			
3A	0.5	0.95	38			
4A	1.0	0.90	25			
5A	2.0	0.80	14			
6A	4.0	0.60	10			
7A	—	1.05	4			
1	0.1ml plasma	0.90	35	$\bar{1}$.78	0.60	6.0
2	0.1ml plasma	0.90	33.5	$\bar{1}$.80	0.63	6.3

ASSAY FLOW SHEET

1.0 ml Standard HGH Solution

or

unknown plasma sample

1:10

+

50 µl anti-HGH Serum

1:8000

↓

mix and incubate at 4°C for 3 days

↓

50 µl (125-I) HGH

2 ng/ml

↓

mix and incubate at 4°C for 3 days

↓

50 µl Rabbit serum (1:100)

50 µl Donkey ARS (1:5)

↓

mix and incubate at 4°C for 24 h

↓

1.0 ml diluent and centrifuge at 4°C, 2300 rpm

for 30 min

Decant supernatant, count precipitate for 2 min.

Some observations on the precipitation in the double-antibody

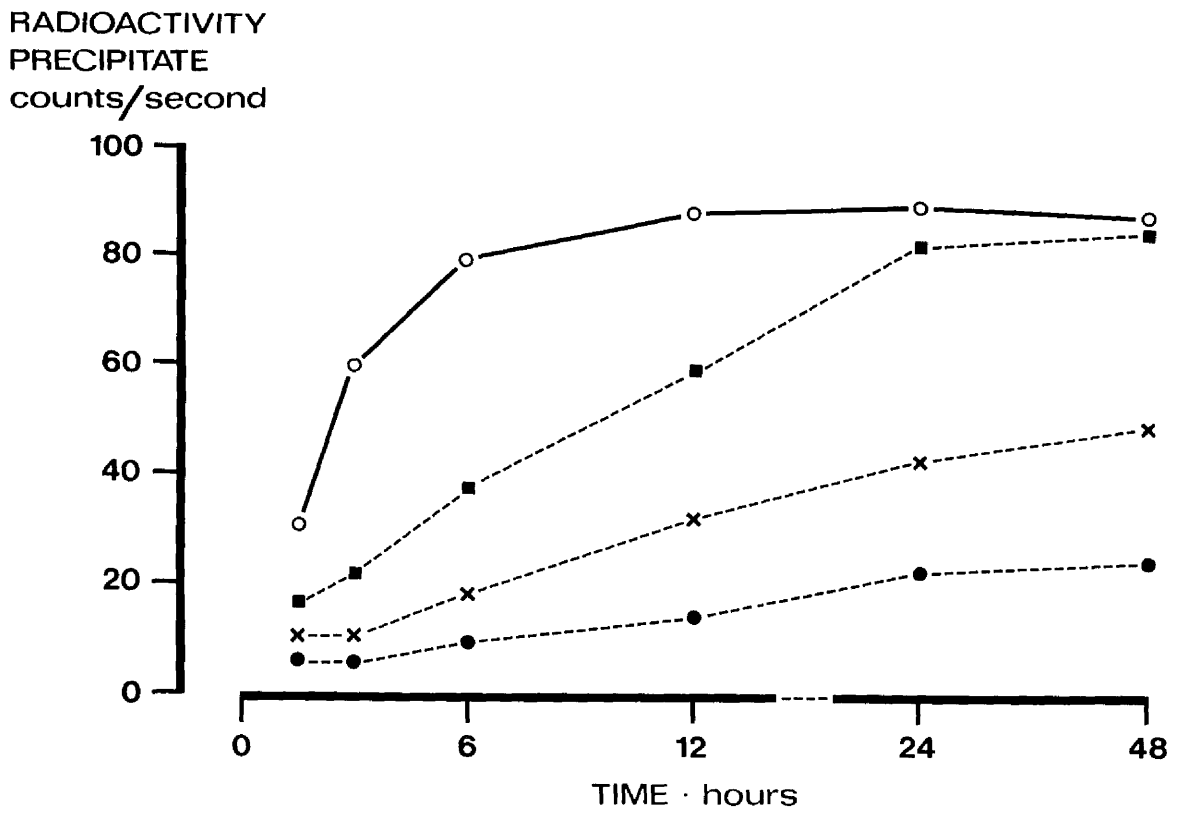
Immuno-assay for HGH

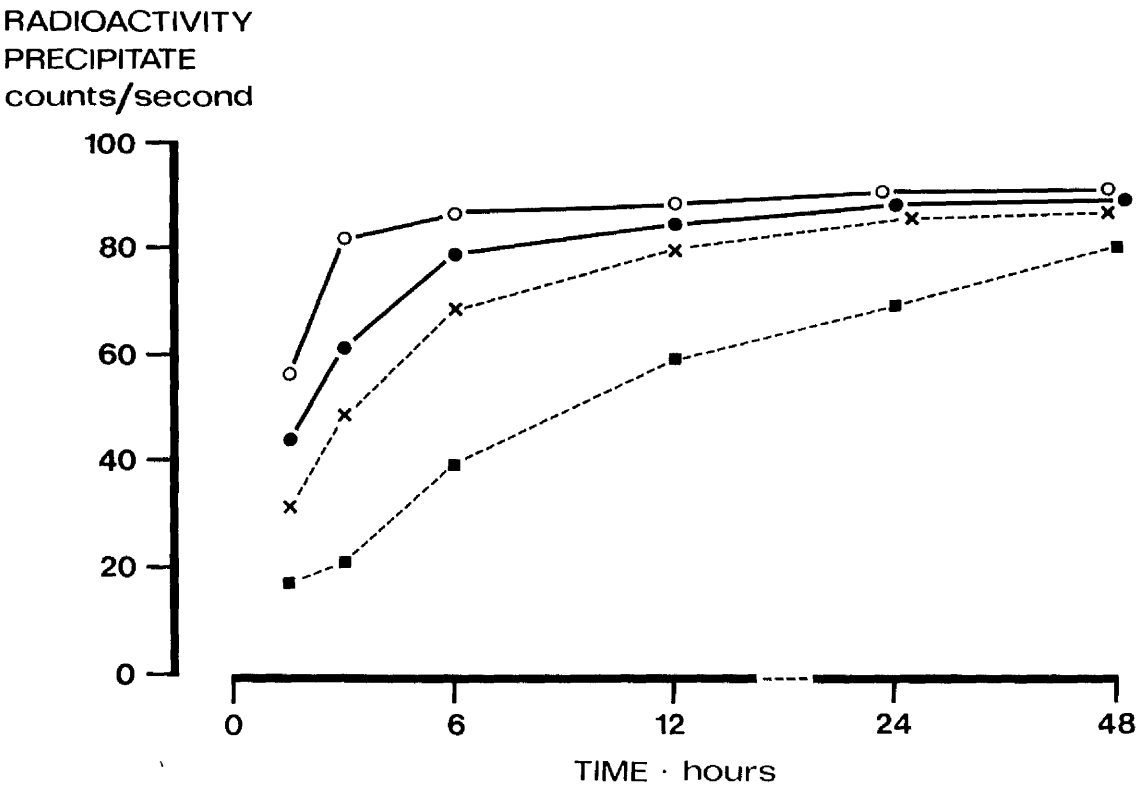
1. The effect of human plasma (Fig 1): when short precipitation periods were used, human plasma consistently reduced precipitate radioactivity to below the levels found in the controls. This effect was less marked after longer periods of precipitation. Increasing the concentration of the second antibody (D-ARS) in the system led to more rapid precipitation of bound hormone, but even under these conditions human plasma delayed precipitation to some extent.

2. The effect of Ethylene diamine tetracetic acid (EDTA) (Fig 2): in the absence of human plasma EDTA was found to accelerate the precipitation of antibody bound HGH. EDTA was also found to increase the radioactivity of the precipitate in the presence of human plasma.

Fig (1): Precipitation of bound (^{125}I) HGH by 50 µl of DARS 1:5 in the presence of 1 ng of unlabelled HGH in diluent (0); and 1 ml volume of undiluted human plasma (●); 1:5 dilution of human plasma (X); 1:10 dilution of human plasma (▣).

Fig (2): The effect of 0.01M EDTA on the precipitation of (^{125}I) HGH by 50 µl of DARS 1:5 in the presence of 1 ng of unlabelled HGH in diluent (0); diluent + EDTA (⊛) and 1 ml of 1:10 dilution of human plasma (▣); 1 ml of 1:10 dilution of human plasma + EDTA (X).


RADIOACTIVITY
PRECIPITATE
counts/second
100
80
60
40
20
0
0
6
12
24
48
TIME · hours


RADIOACTIVITY
PRECIPITATE
counts/second
100
80
60
40
20
0
0
6
12
24
48
TIME · hours

RADIO-IMMUNO ASSAY FOR INSULIN (I I)

Since the original method was reported that charcoal coated with albumin can almost instantly absorb free insulin (Herbert et al., 1965), many developments have been described. Variable non-specific factors may influence assay results particularly the effect of protein and the volume of plasma sample in the incubation mixture when low hormone concentrations are under study.

The procedure for first and second incubation in the estimation of IRI (standards and unknown plasma samples) is very similar to that of HGH, except that albumin pre-treated charcoal is used for the separation of free from bound insulin as described by Hunter and Ganguli (1971), with minor modification.

Reagents

1. Phosphate buffer pH 7.4-7.5 (concentrated stock solution)
   This is made up by dissolving 7.8 g of $NaH_2pO_4 - 2H_2O$ and 101.15 g of Na_2HpO_4 in 500 ml of hot distilled water. The volume is then made up to one litre and stored at room temperature.

2. Phosphate/albumin buffer pH 7.4
   This is made up by dissolving 0.6 g of bovine serum albumin in 200 ml of phosphate buffer diluted 1:4 with distilled water.

3. Human insulin standard (MR71), Wellcome
   The stock solution is stored at a concentration of 1 mU/10 µl in plastic tubes in dilute HCl (N/100). At two monthly intervals

one tube is opened and the contents diluted with dilute HCl containing 0.25% bovine serum albumin to 100 μU/ml, divided into 100 μl aliquots and stored at -20°C. One such tube is opened for each assay.

4. Guinea pig anti-pork insulin antiserum (MT41) Wellcome
The content of each bottle (0.5 ml of 1:1000) is diluted to 1:20,000 with phosphate albumin buffer.

5. [^{125}I] Insulin (IM38), Radiochemical Centre, Amersham, England
With a specific activity of approximately 50 μCi/μg is diluted to a concentration of 10 ng/ml with phosphate albumin buffer and stored in plastic tubes at -20°C. One such tube is opened and used at a concentration of 1 ng/ml.

6. Charcoal Slurry: 1 g charcoal Norite Nk is suspended in 0.3 g/100 ml bovine serum albumin/phosphate buffer pH 7.4 and mixed with a magnetic stirrer for 30 min. 1 ml of this suspension is added to each tube.

7. Plasma samples: the samples are treated as previously described for HGH assay.

ASSAY FLOW SHEET

1.0 ml standard insulin solution

or

unknown plasma sample

1:10

+

50 µl anti-insulin serum

1:20000

↓

mix and incubate at 4°C for 3 days

↓

50 µl (125-I) insulin

1 ng/ml

↓

mix and incubate at 4°C for 3 days

↓

1.0 ml charcoal slurry

↓

mix for 1 min and centrifuge at 4°C, 2300 rpm for 30 min. Decant supernatant, wipe inside carefully with absorbent paper and count for 2 min.

Validity of the Method

The charcoal-coated albumin method for assay of IRI was assessed as follows (1-2). It was also compared with the double antibody technique (3).

1. Assay of known amounts of insulin in increasing dilution of human plasma:

The results of assays carried out on increasing dilutions of the same plasma (originally containing 50 μU/ml) are shown in Table 2. Good agreement was found between the results at different dilutions but the sensitivity of the method decreased using plasma volumes above 0.5 ml similar to the observations with HGH (p . 144).

Table 2 : Insulin assayed in dilutions of a single plasma

plasma dilution	1/20	1/10	1/5	1/2
Insulin μU/ml	48.30	47.80	47.10	40.30
± SEM	± 0.92	± 1.02	± 1.58	± 2.15
n	3	3	3	3

2. Recovery of known amounts of insulin added to human plasma:

Human plasma samples were assayed after the addition of 2.5-40 μU of human insulin/ml by the albumin coated-charcoal method and by the double antibody technique. The results tend to be higher by the double antibody technique than by the coated charcoal method (table 3).

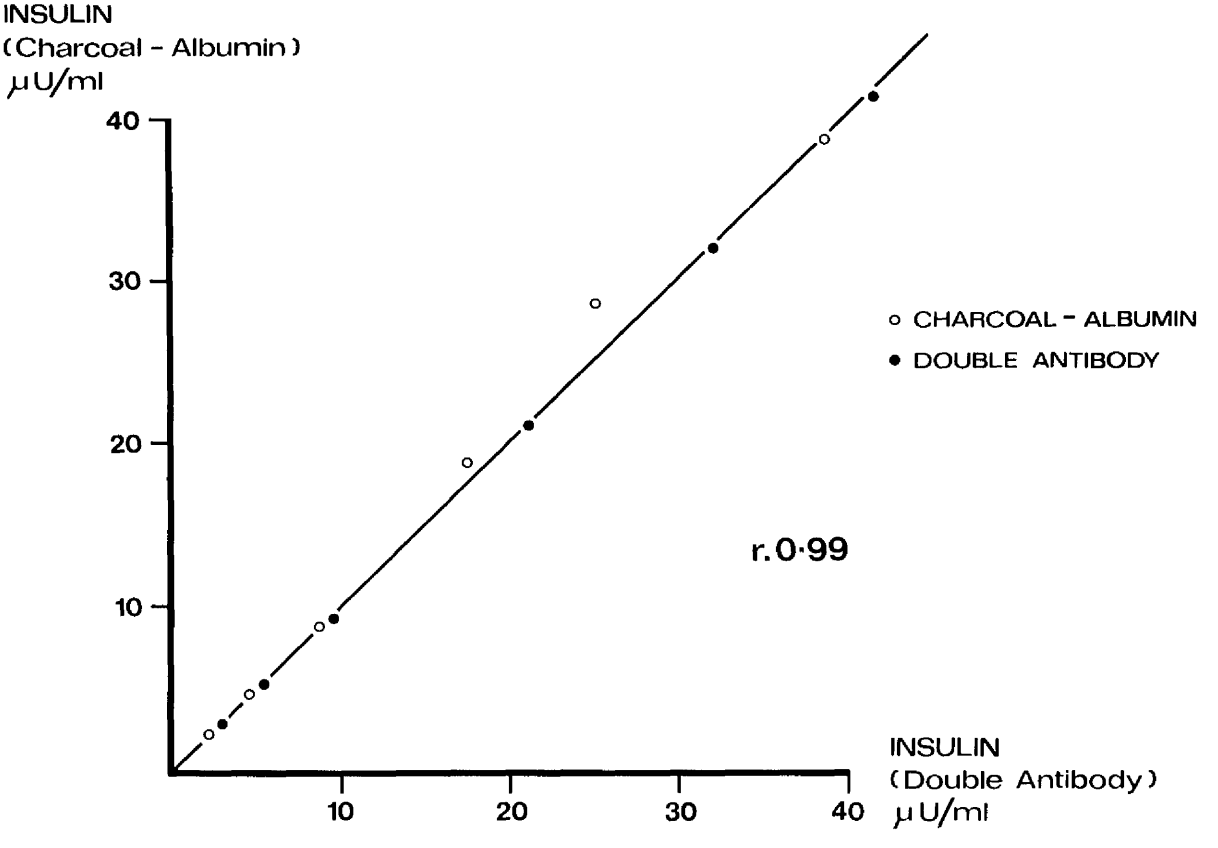
Table 3 Recovery of insulin added to human plasma

		μU insulin added					
Insulin uU/ml		2.5	5.0	10	20	30	40
μU insulin recovered							
Charcoal-albumin technique	100 μl plasma	2.2	4.3	8.8	18.8	28.4	38.2
	in duplicate	2.3	4.5	9.0	18.6	28.7	38.5
Double-antibody technique	100 μl plasma	2.8	5.3	9.6	21.3	32.0	41.6
	in duplicate	3.0	5.3	9.2	21.8	32.0	42.0

3. Comparison of two assay procedures:

A series of plasma samples were assayed by both the albumin coated charcoal method and the double antibody technique. An excellent linear correlation was found between the two techniques (r:0.99) (Fig 3).

Fig (3): Linear correlation between charcoal-albumin technique and the double antibody technique (r. 0.99).


INSULIN
(Charcoal - Albumin)
μU/ml
40
30
20
10
10
20
30
40
INSULIN
(Double Antibody)
μU/ml
○ CHARCOAL - ALBUMIN
● DOUBLE ANTIBODY
r.0·99

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PUBLICATIONS resulting from work reported in this thesis

Presentations (given personally)

1. Extraocular myopathy: abnormal structure and function.
   Doyle, D., Johnson, R.H. and Sulaiman, W.R.
   British Neuropathological Society meeting, June, 1973.

2. Metabolic and hormonal changes in patients with obesity before, during and after starvation in response to exercise.
   Sulaiman, W.R. and Johnson, R.H.
   Exercise Physiology Group meeting, July, 1973.

3. Hormonal and metabolic studies of the action of fenfluramine in normal man, obesity, acromegaly and diabetes mellitus.
   Sulaiman, W.R., Johnson, R.H. and Rennie, M.J.
   Symposium on Fenfluramine and Derivatives, March, 1974.

Communications

1. Sulaiman, W.R., Johnson, R.H. and Rennie, M.J. (1972)
   Blood metabolite changes during fenfluramine therapy compared with partial starvation.
   Clin. Sci. 42; 5P.

2. Sulaiman, W.R., Johnson, R.H. and Park, D.M. (1972)
   The effect of fenfluramine on human growth hormone release.
   Scot. med. J.17; 377.

3. Rennie, M.J., Johnson, R.H. and Sulaiman, W.R. (1972)
   Hormonal and metabolic changes during exercise by patients with acromegaly.
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4. Rennie, M.J., Johnson, R.H., Park, D.M. and Sulaiman, W.R. (1973)
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Clin. Sci. 45; 5P.

5. Rennie, M.J., Sulaiman, W.R., Johnson, R.H. and Park, D.M. (1974)
Hormonal responses to exercise in racing cyclists.
J. Physiol. (in press).

Papers

1. Johnson, R.H., Sulaiman, W.R. and Webster, M.H.C. (1972)
Human growth hormone and ketosis in athletes and non-athletes.
Nature 236; 119 - 120.

2. Sulaiman, W.R. and Johnson, R.H. (1973)
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3. Sulaiman, W.R., Doyle, D., Johnson, R.H. and Jennet, S.
Myopathy with mitochondrial inclusion bodies; histological and metabolic studies. (Completed - about to be submitted to J. Neurol. Neurosurg. & Psychiat.).