PHYSIOLOGICAL AND BIOCHEMICAL CHANGES WITH EXERCISE

by

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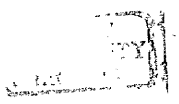

ACKNOWLEDGEMENTS

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Michael Rennie
October 1973.

PHYSIOLOGICAL AND BIOCHEMICAL CHANGES WITH EXERCISE

CONTENTS

ABBREVIATIONS USED IN THIS THESIS

A	-	adrenaline
BTPS	-	body temperature and pressure (saturated)
ATP	-	adenosine triphosphate
BSA	-	bovine serum albumin
e.c.g.	-	electrocardiograph
FFA	-	free fatty acid
HGH	-	human growth hormone
IRI	-	immunoreactive insulin
M	-	mol or molar
N	-	normal
NA	-	noradrenaline
NAD (NADH)		nicotinamide adenine dinucleotide (reduced)
PCA	-	perchloric acid
R	-	respiratory exchange ratio
STPD	-	standard temperature and pressure (dry)
THI	-	trihydroxyindole
UV	-	ultraviolet
VCO_2	-	CO_2 output STPD
VO_2 max	-	maximal oxygen uptake STPD

Units used

l	-	litre	
ml	-	millilitre	- 10^{-3}l
ul	-	microlitre	- 10^{-6}l
g	-	gram	
mg	-	milligram	- 10^{-3}g
ug	-	microgram	- 10^{-6}g
ng	-	nanogram	- 10^{-9}g

nm	-	nanometre	- 10^{-9}m
µ mol	-	micromol	- 10^{-6}mol

INTRODUCTION

(a) Historical background

Recent history contains many examples of man's increasing technological power over his surroundings. Nevertheless, the body as a machine for converting the energy of food into force and work remains his primary means of changing the world around him.

The realisation that human work depends on food must be very old, but the scientific examination of their relationship was only possible when the practice of physics and chemistry became sufficiently precise. Two lines of enquiry into the workings of muscles may be traced from the 18th century.

The first sprang from the increasing understanding of the physics of heat and of gases and the central importance of combustion. Lavoisier and Laplace first studied the connection between work and respiration and measured oxygen consumption during exercise by one subject before and after feeding. However, it was German physiologists working in the middle of the 19th century who gave the great impetus to the study of energy metabolism by their application of the new doctrine of the conservation of energy. Their methods of physiological thermodynamics and respiratory-gas measurement have since been developed as tools in the appraisal of the energy economy of the intact individual at rest and during exercise.

The other approach, which derived from microscopical studies, was centred around Schwann's general theory of the cell as the basis of all life. However, Schwann's units of life were

each thought to have their own separate life and metabolism. The disentangling of cellular physiology and metabolism gradually became possible under Liebig's influence as a practical physiological chemist and the school of electrophysiology deriving from Helmholtz.

Earlier this century work on the function of the isolated preparation of animal muscle was divided into the investigation of the electrical and mechanical processes of contraction, and the study of metabolic processes accompanying them. These two lines rejoined in 1924 when A.V. Hill integrated his thermodynamic studies of the nerve-muscle preparation with Fletcher's and Hopkin's observations on glycogen breakdown and lactate build-up in muscular work. The integration became more complete with the Eggletons' discovery in 1927 of energy rich phosphagens and the later indentification (1939) of these with ATP as the universal cellular energy mediator by Lohman, Fiske and Subbarrow. The work of Lohman and Englehardt, and of Szent-Gyorgi twenty years later, on the ATP-splitting properties of myosin, lead directly through Huxley's elegant electron-microscopy of muscle to Davies' molecular theory of contraction (1963).

In the years since the war there has been a movement to reintegrate the biochemical and cytological knowledge of muscle structure and function with the physiology of whole-body energy metabolism in exercising man. The principal advances have been made in the development of techniques for accurate, sensitive and precise measurement of the blood concentration of metabolites. Recent techniques of muscle biopsy have extended the range of study still further and together these tools have

enabled exercise physiologists to examine the sources of stored fuels for exercise, the way in which the fuel is transported and many of the steps in the energy metabolism of working muscle.

(b) Fuel for muscular work - the carbohydrate/fat controversy

Within thirty years of Mayer's application of the first law of thermodynamics to physiology, von Voit and Pettenkofer had assigned energy values to the now classical categories of food-stuffs - carbohydrate, fat and protein, and Rubner had measured changes after their consumption by a technique of whole-body calorimetry. Various workers measured respiratory gas exchange during exercise, including Chaveau in France who used a work regime resulting in exhaustion within an hour. He found the respiratory quotient at the end of exercise to be unity indicating carbohydrate as the exclusive muscle fuel. German workers, however, led by Zuntz, used a low-level exercise regime which could be sustained for long periods and showed that the RQ either remained steady at the resting value or fell, indicating the use of fat as fuel.

The Zuntz-Chaveau controversy was not solved until Christensen and Hansen published results (1939) which indicated that in light to moderate exercise of short duration either fat or carbohydrate was used depending largly on diet. In longer duration moderate exercise the proportion of fat utilised increases gradually due to reduction of carbohydrate stores. In exercise of increasing intensity the contribution of carbohydrate utilisation to energy production becomes more pronounced until it reaches 100% at maximal work loads.

Many problems remained however. How was the change-over controlled? Was muscle, blood or liver the source of the fuel used? What was the exact nature of relationship between oxygen consumption and use of carbohydrate or fat as fuel? Was anaerobic metabolism or aerobic metabolism more important during exercise? Some of the questions are very old and only partial answers have been found for them.

(c) A biochemical approach to fat metabolism during and after exercise

Since lactate builds up in the muscle cells during muscular work and is then discharged into the blood, it had become almost traditional, despite the work of Christensen & Hansen, to regard the metabolic changes accompanying prolonged submaximal exercise (such as middle distance running) as consisting mainly of the production of lactate by glycolysis from carbohydrate with the fully oxidative processes of muscle mitochondria contributing little, despite their greater potential efficiency. It was previously thought that the changeover to glycolysis was due to a fall in oxygen concentration in the muscle cells, so slowing the mitochondrial oxidation processes. However, it seems that mitochondrial exidation can take place at very low concentrations of oxygen and that the bottleneck hindering the through-put of fuels in mitochondria lies in the production from pyruvate of a high energy derivative of acetic acid, i.e. acetyl coenzyme A (acetyl Co-A). The further oxidation of acetyl Co-A takes place in the mitochondria by the Krebs cycle.

It seems, then, that if acetyl Co-A could be produced from a source other than muscle glycogen the bottleneck at pyruvate

oxidation might be by-passed and the more efficient processes of oxidative phosphorylation continue. The oxidation of fatty acids provides such a mechanism whereby two carbon containing fragments of long chain fatty acids are broken off from the chain with the release of electrons to mitochondrial oxidising agents. Each two-carbon fragment is combined with co-enzyme A to form a further source of energy within the mitochondria - acetyl Co-A. However, although this mechanism is capable of providing energy for work the store of fat in muscle, and the ability of muscle to take up circulating fatty acids is limited. This difficulty is overcome by the capacity of the liver to oxidise fatty acids to acetyl Co-A and then combine units of acetyl Co-A into four-carbon containing compounds which are easily transported in the blood and may be used as fuel by the working muscle. These compounds, ketone-bodies (acetoacetate and 3-hydroxybutyrate), accumulate in the blood whenever their formation after the breakdown of fatty acids exceeds their utilisation.

Recent work indicated that trained runners were able to adjust their metabolic economy to an efficient usage of fat via ketone-body oxidation in muscles.

That this was so was suggested by two chance observations. Firstly that fit but by no means athletically trained hill walkers developed high concentrations of ketone-bodies in their urine after a day on the fells (Pugh, 1969) and secondly that marathon runners showed only small amounts of ketone-bodies in their urine after finishing a race (Pugh, Corbett & Johnson, 1967). This might have been explained by the differences in fat breakdown or utilisation.

An investigation of metabolic changes in the blood of athletes and non-athletes during 1½ hours of running showed that fat (as triglycerides i.e. compounds of fatty acids and glycerol) was broken down into glycerol and free fatty acids in both groups but that there was no accumulation of ketone-bodies after exercise in the athletes (Johnson, Walton, Krebs & Williamson, 1969). The non-athletes, however, showed considerable post-exercise ketosis, perhaps due to an imbalance between the rate of production of ketone-bodies as fuel and their utilisation.

When a solution of acetoacetate was given orally to a group of athletes and non-athletes at rest there was no apparent difference in the rate of disappearance of the substance from the blood but during and after exercise there were marked differences between the two groups (Johnson & Walton, 1972). The non-athletes showed a decreased tolerance to acetoacetate reflected in greater blood levels, while the trained group seemed able to handle ketone-bodies as well during and after exercise as before. This showed an apparent ability on the behalf of athletes to increase the availability of fuel to the oxidative processes producing ATP in the mitochondria and to utilise such fuels efficiently.

(d) The present work

What factors might regulate the more efficient utilisation of fat for fuel in strenuous muscular work? The studies presented in this thesis approached this problem in the following ways.

First, were the observations previously made during and after exercise repeatable? How did the intensity and duration of exercise change patterns of observed changes in metabolites with exercise? Second, did the differences previously observed in blood metabolite concentration between athletes and untrained subjects reflect a relatively greater level of work by the untrained subjects, with the athletes showing smaller changes in fat metabolites because they paced themselves more effectively? Could similar results be obtained by athletic training in a group of untrained subjects?

Diet is of great subjective importance to athletes and Scandinavian workers had shown how muscle glycogen stores could be increased by a suitable exercise and diet regime (Hermansen, Hultman & Saltin, 1967). Could diet change the ways in which circulating fat and carbohydrate fuels are used by trained runners?

The whole-body metabolism of fat and carbohydrate are well known to be affected by changes in hormonal activity. How, therefore, did hormones affect the supply and utilisation of muscular fuels? There was little information available on the pattern of release of hormones during exercise. Insulin had been reported both to fall (Devlin, 1963) and rise (Nikkila, Taskinen, Miettinen, Pelkonen & Poppius, 1968) but there had been little systematic investigation. What factors influenced insulin secretion during exercise? Similar confusion existed in the literature on the nature of the response to exercise of cortisol secretion with some reports of a rise (Chinn & Evonuk, 1971) and others of a fall (Frenkl & Csalay, 1962). Studies were therefore designed to discover the nature of the true

responses to a controlled work regime.

Catecholamines have long been known to rise under conditions of fight or flight but the exact relationship between absolute and relative work load and secretion of both adrenaline from the adrenal cortex and noradrenaline from sympathetic nerve terminals remained poorly understood. The influence of athletic fitness on catecholamine secretion was also unknown because of lack of basic knowledge of the changes during exercise. Many of the problems in examining changes in levels of catecholamines were due to severe methodological difficulties. Techniques were therefore developed for differential assay of small amounts of adrenaline and noradrenaline in blood and the improved procedures applied to exercise studies.

The patterns of hormonal regulation do appear to be somewhat modified in trained athletes. For example, they secrete less human growth hormone during exercise than non-athletes (Sutton, Young, Lazarus, Hickie & Maksvytis, 1968). It was possible that differences in growth hormone secretion during exercise might explain observed differences between athletes and non-athletes in post-exercise ketosis. The opportunity to examine the influence of growth hormone on metabolic changes with exercise was provided within the University of Glasgow Department of Neurology by the study of patients suffering from pituitary tumours, which could cause either an excess or a lack of human growth hormone. In the same way that an increased understanding of normal physiological processes should be capable of application to the study of the abnormal processes in disease states, then certain specific disorders may provide unusual model situations for the study of altered

physiological processes. Thus the investigation of metabolic changes with exercise in patients suffering from hypopituitarism and acromegaly was likely to provide information on the role of growth hormone in fat metabolism.

As more experience of normal exercise physiology and biochemistry was accumulated it became possible to apply the results directly to neurological problems. This approach was shown to be worthwhile in a study of the inappropriate fatigue developed with exercise by patients with no physical signs and also in a study of the metabolic changes occurring with exercise in patients with muscular dystrophy.

This thesis presents the results of investigation of some factors which change the metabolic response to exercise and also examines the altered responses in a number of neurological diseases.

Sources used for this introduction:-

FRANKLIN, K.J. (1949) A Short History of Physiology. Staples Press, London.

MORHOUSE, L.E. & MILLER, Jr. A.T. (1967) Physiology of Exercise. C.V. Mosby Co.

PERNOW, P. & SALTIN, B. (Eds) (1971) Muscle Metabolism during Exercise. Plenum Press, New York.

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References given in section c & d are quoted in full at the end of this thesis.

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.

SUMMARY OF APPENDIX I

(a) Physiological techniques: The type of exercise employed in the investigations described in the following chapters was varied according to the aims of each study. Subjects have run for 1 and 1½ hr and have pedalled a bicycle ergometer at both steady and increasing work loads for periods of up to 40 min.

Heart rate was recorded from an electrocardiogram using lead system II. Ventilation and gas-exchange measurements have been made in some studies by Dr. Sheila Jennett and these are described in the appropriate chapter.

Blood samples were taken either by venepuncture or via an indwelling catheter placed in an arm vein.

(b) Biochemical techniques: Blood samples (14 ml) were divided into two aliquots. A 4 ml portion was deproteinised with perchloric acid. The remainder was heparinised and the blood cells were separated from plasma by centrifugation. The neutralised deproteinised blood extract was used for estimation of metabolites. Lactate, pyruvate, acetoacetate, 3-hydroxybutyrate and glycerol were estimated by enzymatic methods using the absorbance of NADH in UV/light at 340 nm. Glucose was measured by colorimetric method using glucose-oxidase-peroxidase and an organic dye as a redox indicator.

The plasma was used for the estimation of FFA by formation of a copper soap and the colorimetric estimation of this by use of a specific copper reagent.

Insulin and growth hormone were also estimated on an aliquot of the plasma by immunoreactive techniques. Plasma cortisol was measured by a modification of a standard fluorimetric technique using sulphurid acid/ethanol for the formation of a fluorophor.

SUMMARY OF APPENDIX II

A technique has been developed for analysis of small quantities of catecholamines in plasma. A modified method has been evaluated carefully.

Blood samples (20 ml) for catecholamine analysis were transferred immediately to heavy glass tubes in ice-water. The tubes contained a mixture of EDTA, sodium metabisulphite and pargyline to prevent clotting and losses of amines by oxidation and metabolism in blood.

Catecholamines were separated and purified by adsorption on alumina and subsequent ion-exchange chromatography. Differential assay of adrenaline and noradrenaline was performed by a spectrofluorimetric method after formation of fluorophors using the trihydroxyindole reaction.

CHAPTER 1

THE EFFECTS OF INTENSITY AND DURATION OF EXERCISE ON THE METABOLIC CHANGES

1.1 INTRODUCTION

It is now known that working skeletal muscle is able to use both stored fuel (as glycogen and possibly triglycerides) and fuel supplied via the circulation as glucose, FFA and ketone-bodies. The proportions of the fuels used from these sources and the amounts of carbohydrate and fat-derived substances oxidised in muscular work depends upon the intensity of the work and its duration (Christensen & Hansen, 1939; Havel, 1971).

The relationship between the amount of the fuels mobilised into the circulation as a result of exercise of altered intensity and duration and the changes in the recovery period after exercise is less well understood. Much work has been previously carried out on the relationship of the oxygen debt to blood levels of lactic acid but many of the conculusions reached have depended upon a poor understanding of the underlying biochemical mechanisms (Harris, 1969). Hardly any study has been made of the relationships between changes in plasma FFA during exercise and the development of post-exercise ketosis. The present study was designed first to investigate previously reported changes during and after exercise in metabolites in the blood (Johnson et al., 1969; Young, Pelligra & Adachi, 1966). Second, the relationships of these changes to differences in work intensity and duration were to be studied.

1.2 PROCEDURE

Five volunteers (men aged 22-28, 166-178 cm, 75-94 kg) agreed to take part in a programme of exercise on a bicycle ergometer. Each subject exercised for 20 min on three occasions

TABLE 1:i

(a) Intensity Study

Subject	Work rate kpm/min		
	Low	Moderate	Severe
A.B.	500	800	1100
W.H.	500	750	1000
J.M.	500	800	1100
M.R.	600	900	1200
B.J.	600	900	1200

All subjects exercised for 20 min.

(b) Duration Study

Subject	Minutes of exercise at 900 kpm/min		
M.J.	20	30	40
B.J.	20	30	40

at low, moderate and severe work loads (see Table 1:i). On two additional occasions two of the subjects exercised at the intermediate work load for 30 and 40 min. Blood samples were taken at rest, at 5 min intervals during the period of exercise and at 5 30 min intervals afterwards by means of an indwelling catheter placed in an arm vein. The samples were analysed for glucose, lactate, pyruvate, acetoacetate, 3-hydroxybutyrate, glycerol and plasma FFA. Heart rate was recorded using a continuous e.c.g.

1.3 RESULTS

1.3.1 THE EFFECT OF DIFFERENT INTENSITIES OF EXERCISE

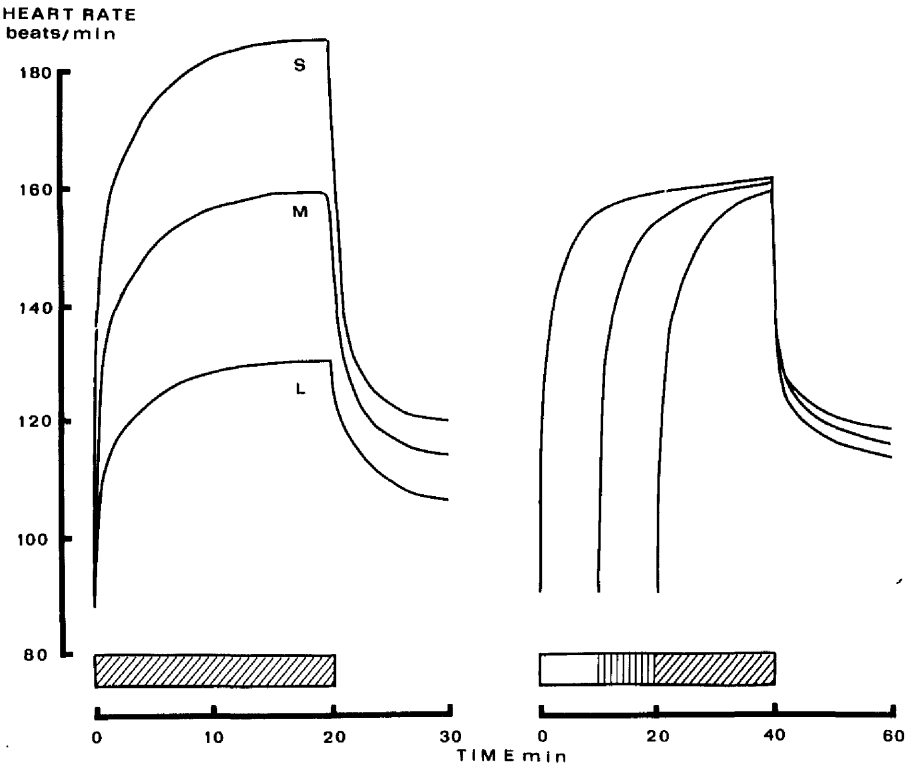
(a) Heart rate: (Fig. 1:i) Heart rate increased directly with increase of work load and increases in heart rates were proportional to the increase in exercise intensity.

(b) Glucose, lactate and pyruvate: (Fig. 1:ii) The concentrations of these metabolites increased during the period of exercise at a progressively greater rate with increased work loads.

The lactate and pyruvate concentrations tended to fall before the end of exercise at the low intensity loads but rose progressively at the highest load and in three subjects were highest 5 min after the end of exercise.

Glucose concentrations fell slowly after exercise but there was a rapid decay in levels of lactate and pyruvate. The rates of recovery of lactate and pyruvate (μ mol/ml/min) were greatest after the most strenuous exercise.

Fig. 1:i Mean heart-rate changes during and after 20 min exercise of low, moderate and severe intensity (5 subjects); and during and after 20, 30 and 40 min of moderate exercise (2 subjects).


HEART RATE
beats/min
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20
40
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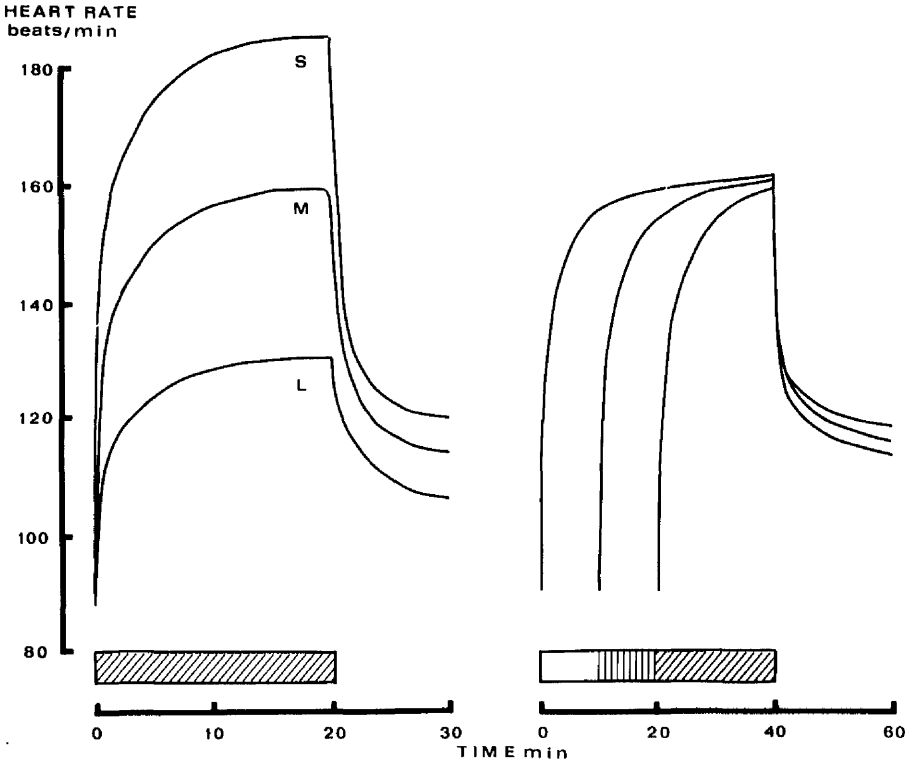
TIME min


HEART RATE
beats/min
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160
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20
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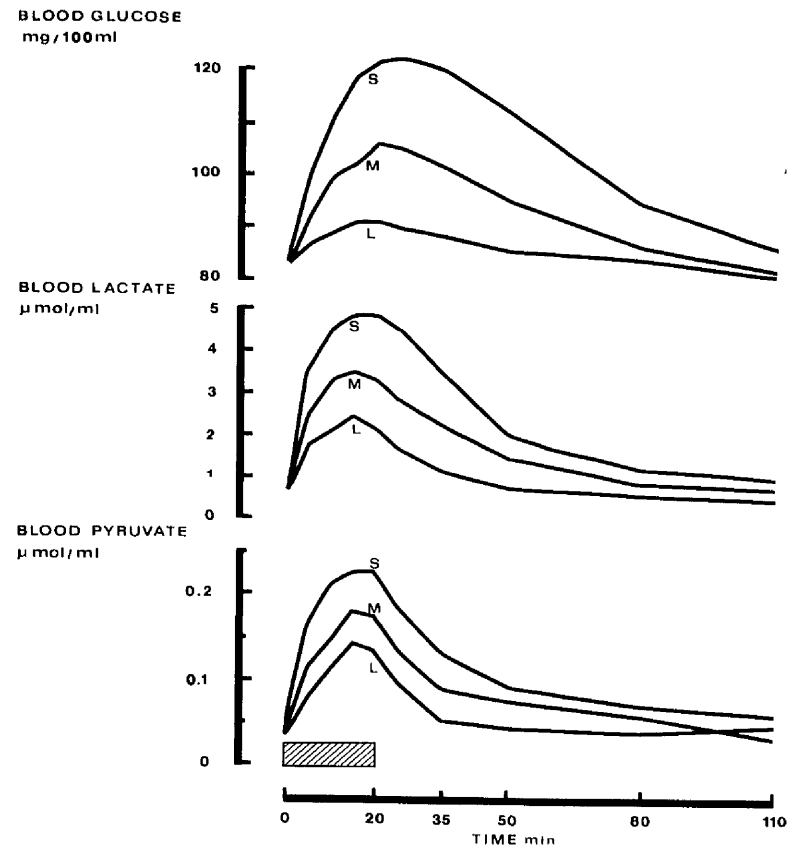
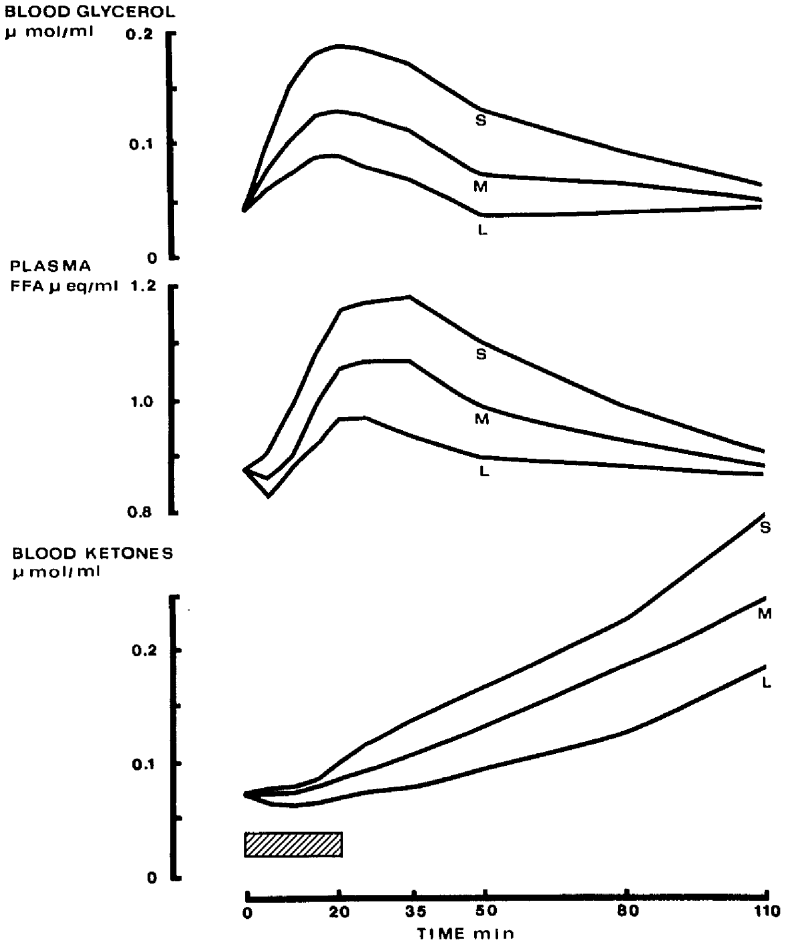
TIME min

Fig. 1:ii Mean changes in blood glucose (mg/100 ml), blood lactate (μmol/ml) and blood pyruvate (μmol/ml) during and after exercise of low, moderate and severe intensity in 5 subjects.

Fig. 1:iii Mean changes in blood glycerol (μmol/ml), plasma FFA (μequiv/ml) and blood ketone-bodies (μmol/ml) during and after exercise of low, moderate and severe intensity in 5 subjects.


BLOOD GLUCOSE
mg/100ml
120
100
80
S
M
L
BLOOD LACTATE
µmol/ml
5
4
3
2
1
0
BLOOD PYRUVATE
µmol/ml
0.2
0.1
0
0
20
35
50
80
110
TIME min


BLOOD GLYCEROL
µ mol/ml
0.2
0.1
0
PLASMA
FFA µ eq/ml
1.2
1.0
0.8
BLOOD KETONES
µmol/ml
0.2
0.1
0
S
M
L
0
20
35
50
80
110
TIME min

(c) Glycerol, FFA and ketone-bodies: (Fig. 1:iii)

FFA levels fell significantly at the beginning of exercise at low and moderate work loads ($P < 0.05$). Thereafter glycerol and FFA concentrations rose throughout the exercise period at all work loads but the increases were greatest at the highest working intensity. Blood ketone-bodies fell slightly during the first 5 min of exercise at low and moderate work loads. During the remainder of the exercise they rose slightly above resting values. The magnitude of the changes were broadly related to the severity of the exercise. After exercise the blood concentrations of ketone-bodies rose progressively.

1.3.2 THE EFFECT OF DURATION OF EXERCISE

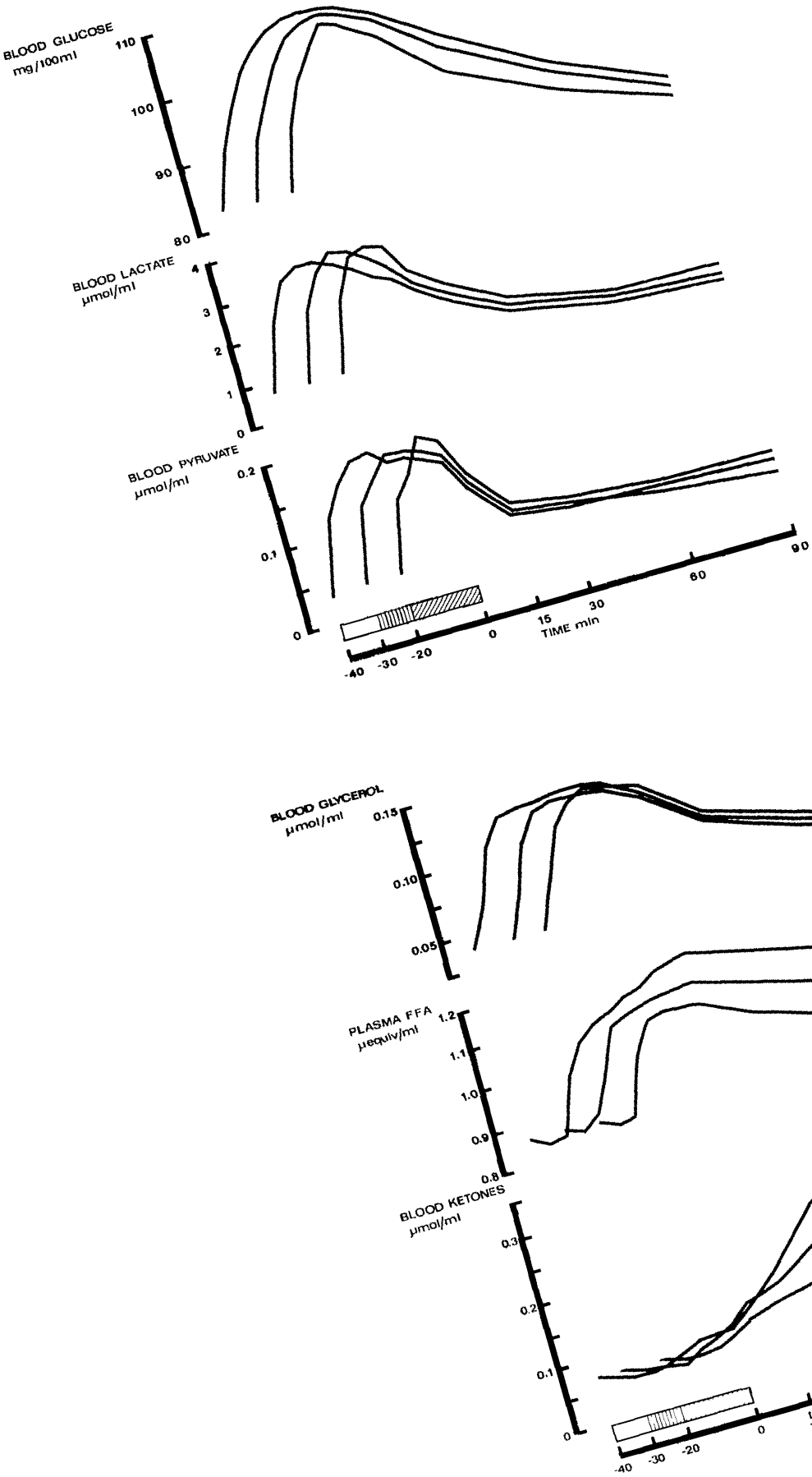
(a) Heart rate: (Fig. 1:i) Heart rate increased at the same rate during the first 15 min of exercise on each occasion. There was a further small increase so that the heart rate after 30 and 40 min of exercise was slightly greater than the 20 min rate.

(b) Metabolites: (Fig. 1:iv & 1:v) Glucose concentration was greater after 30 and 40 min of exercise at 900 kpm in two subjects than after 20 min but both lactate and pyruvate concentrations fell slightly during the longer periods of exercise. Blood glucose continued to increase at 5 and 15 min after the longer exercise periods but then fell in the remainder of the investigations. Lactate and pyruvate fell immediately after exercise.

Glycerol levels did not increase throughout the longer periods of exercise but there was a continued increase in plasma FFA after exercise of longer than 30 min. The post-exercise

Fig. 1:iv Mean changes in blood glucose (mg/100 ml), blood lactate (μmol/ml) and blood pyruvate during and after 20, 30 and 40 min of exercise at 900 kpm/min in 2 subjects.

Fig. 1:v Mean changes in blood glycerol (μmol/ml), plasma FFA (μequiv/ml) and blood ketone bodies (μmol/ml) during and after 20, 30 and 40 min of exercise at 900 kpm/min in 2 subjects.


BLOOD GLUCOSE
mg/100ml
110
100
90
80
BLOOD LACTATE
µmol/ml
4
3
2
1
0
BLOOD PYRUVATE
µmol/ml
0.2
0.1
0
-40
-30
-20
0
15
30
60
90
TIME min
BLOOD GLYCEROL
µmol/ml
0.15
0.10
0.05
PLASMA FFA
µequiv/ml
1.2
1.1
1.0
0.9
0.8
BLOOD KETONES
µmol/ml
0.3
0.2
0.1
0

concentrations of glycerol fell slowly but FFA concentrations rose after the 30 and 40 min exercise tests. The blood ketone-body concentrations rose during the longer periods of exercise. The development of post-exercise ketosis was much greater after 30 and 40 min of exercise than after 20 min.

1.3.3 RELATIONSHIPS BETWEEN METABOLIC CHANGES, WORK LOAD AND WORK TIME (TABLE 1:ii)

A number of possible relationships were tested by regression analysis. Table 1:ii gives details of the most important results. There was a good linear correlation between changes in blood levels of all metabolites and both work load and work time.

The increment in lactate and pyruvate showed a better correlation with work load at 5 min than at 10 and 20 min of exercise.

When the area under the graphs of metabolite concentration change were examined a number of relationships became obvious. The area under the lactate and pyruvate curves during exercise was positively logarithmically correlated to work load. There was a poor relationship between the area after exercise and the work load. The area under the curve during exercise was linearly related to work time for lactate and pyruvate.

Both the increment at the end of exercise and the log. incremental areas during and after exercise under the curves for glucose, glycerol, FFA and ketone-bodies were related to work load. There was also a good correlation between the incremental area under the curves of these metabolites and

Table 1:ii Regression analysis of metabolic changes vs work load (w) and work time (t)

	y	x	y = a x + b	r	n	P
LACTATE	Δ L 5 min (μmol/ml)	w kpm/min	y = 0.0026x - 0.523	0.813	15	0.001
	Δ L 10 min (μmol/ml)	w kpm/min	y = 0.004x - 0.966	0.759	15	0.01
	Δ L 20 min (μmol/ml)	w kpm/min	y = 0.0046x - 1.307	0.573	15	0.05
	Δ L 20 min (μmol/ml)	w kpm/min	log y = 0.0024x - 1.08	0.653	15	0.01
	Δ Exercise Area L (μmol/ml x min)	w kpm/min	log y = 0.0012x + 0.42	0.999	3	0.01
	Δ Exercise Area L (μmol/ml x min)	t min	y = 0.53x + 0.48	0.875	6	0.05
PYRUVATE	Δ P 20 min (μmol/ml)	w kpm/min	y = 0.001x + 0.055	0.822	15	0.001
	Δ P 20 min (μmol/1)	w kpm/min	log y = 0.004x + 1.79	0.758	15	0.01
	Δ Exercise Area P (μmol/1 x min)	w kpm/min	log y = 0.0006x + 2.62	0.987	3	0.10
	Δ Exercise Area P (μmol/ml x min)	t min	y = 0.065x - 0.20	0.825	6	0.05
GLUCOSE	Δ GLU 20 min (mg/100ml)	w kpm/min	y = 0.05x -21.8	0.545	15	0.05
	Δ GLU Total Area (mg/100ml x min)	w kpm/min	log y = 0.0014x + 1.02	0.999	3	0.01
	Δ GLU Total Area (mg/100ml x min)	t min	y = 2.3x +55	0.845	6	0.05
GLYCEROL	Δ GLY 20 min (μmol/ml)	w kpm/min	y = 0.00017x- 0.053		15	
	Δ GLY Total Area (μmol/1 x min)	w kpm/min	log y = 0.00087x+ 2.74	0.999	3	0.01
	ΔGLY Total Area (μmol/ml x min)	t min	y = 0.042x + 1.308	0.948	6	0.01

Key to Table:

Δ = increment

Exercise area = area under curve during exercise

Total area = total area under curve

r = correlation coefficient

n = number of pairs

P = significance limit

Continued....

Table 1:ii (contd)

y	x	y = a x + b	r	n	P
Δ FFA 20 min (μequiv/ml)	w kpm/min	y = 0.0046x - 2.55	0.759	15	0.01
Δ FFA Total Area (μequiv/l x min)	w kpm/min	log y = 0.00075x+ 3.315	0.9992	3	0.01
ΔFFA Total Area (μequiv/ml x min)	t min	y = 0.82x - 8.53	0.844	6	0.05
Δ KB 90 min post-exercise (μmol/ml)	w kpm/min	y = 0.0023x - 0.43	0.512	15	0.1
+Δ Area KB post-exercise (μmol/l x min)	w kpm/min	log y = 0.00077x+ 3.19	0.998	3	0.05
+Δ Area KB post-exercise (μmol/ml x min)	t min	y = 0.47x - 4.21	0.818	6	0.05
ΔFFA (μequiv/ml)	ΔArea KB post-exercise (μmol/l x min)	log y = 0.234x + 3.44	0.998	3	0.05

Key to Table:

Δ = increment

Exercise area = area under curve during exercise

Total area = total area under curve

r = correlation coefficient

n = number of pairs

P = significance limit

duration of exercise at a fixed work load.

1.4 DISCUSSION

The amount of glycogen depletion at the end of exhaustive exercise is dependent on the work load (Hermansen, Hultman & Saltin, 1967). There should therefore be a direct relationship between work load and lactate production by muscle. Such a relationship has been found between blood lactate concentration at the end of exhaustive exercise and work load (Karlsson & Saltin, 1970; Karlsson, Diamant & Saltin, 1971; Margaria, Cerretelli, Aghemo & Sassi, 1963). The present results also show a direct relationship between blood lactate and pyruvate concentration and work load. However, since the rate of increase of blood lactate slowed down towards the end of exercise the correlation was better with both the increment at the end of exercise and the incremental area under the lactate and pyruvate curves during exercise.

The kinetics of lactate accumulation in blood during exercise are very complicated since important factors such as pool sizes and numbers, concentration gradients, blood flow and transport across membranes may all change in a non-linear fashion. Under such conditions the logarithm of the incremental area of lactate change during exercise appears to be a better index of lactate production in sub-maximal non-exhaustive exercise than the blood levels alone, since the correlation is improved when this relationship is examined. The positive correlation between the area under the lactate and pyruvate curves during exercise and the

duration of exercise was linear. This indicates that within the time limits studied at a given submaximal work load the rates of production and removal of lactate reach a steady state. Similar results have been obtained by other workers (Hultman, 1967; Karlsson & Saltin, 1967).

The rate of disappearance of lactate after exercise has previously been shown to be a complex exponential decay (Gisolfi, Robinson & Turrell, 1966). Although the blood level of lactate rose after exercise at the highest intensity the rate of decay was nevertheless greatest under these circumstances. Both of these observations might be explained by a greater post-exercise blood flow through skeletal muscle, which besides releasing lactate may also take it up from the circulation (Jorfeldt, 1970). Although the lactate and pyruvate areas during exercise were related in a linear fashion to increased duration of work, there was a logarithmic correlation with work intensity suggesting that there may be a different relationship under these conditions between power output and lactate production. The situation might be analogous to the exponential decrease of fuel utilisation during the acceleration of a motor car compared to the linear decreast at constant speed.

The rise in blood glucose levels induced by exercise is well known (Wahren, Ahlborg, Felig & Jorfeldt, 1971; Rowell, Masoro & Spencer, 1965). Wahren et al. have demonstrated a positive relationship between work load and arterial glucose concentration and a-v glucose difference. The present results indirectly support these findings and suggest that the increase in blood glucose, measured in venous blood, is also related in

a direct manner to work load. The total glucose production is also apparently related linearly to work time. The increases in blood glucose are mainly the result of increased glycogenolysis in liver and only partly due to increased gluconeogenesis from lactate and glycerol at high energy cost. The observed pattern of work load - glucose relationships suggest that stimulation by circulating catecholamines of hepatic glycogen breakdown may be an important factor in glucose mobilisation. Thus the supply of glucose in the circulation is probably not regulated primarily by working muscle but is determined largely by outside factors.

The present results indicate that mobilisation of stored fat in adipose tissue is also largely controlled by factors secondary to muscular contraction. However, since a smaller proportion of fat than carbohydrate is oxidised at high work intensity, the best relationship between plasma FFA increment and work load is curvilinear. There is also curvilinear relationship between incremental glycerol area and work load but the rate of change is less than for FFA, supporting the idea of proportionate decrease of FFA oxidation at high work loads. At a fixed work load there is nevertheless a linear relationship between glycerol, FFA and work time and thus power output.

The behaviour of ketone-body concentrations in the blood is complex. The initial decrease in ketone-body levels during exercise is probably related to increased peripheral blood flow rather than to uptake by muscle. The a-v difference in ketone-bodies decreases during exercise and a net outflow has been observed from working muscle (Hagenfeldt & Wahren, 1968),

However, the liver is a more likely source even at reduced hepatic flow rates for the increased ketone-body concentrations seen in the present studies. The incremental area of ketone-bodies after exercise was related in a logarithmic fashion to plasma FFA concentration and bore the same relationship to exercise intensity but a linear relationship to exercise duration. It appears, therefore, that post-exercise ketosis is related to excess production of plasma FFA and its removal by liver rather than to imperfect oxidation of FFA by working muscle.

The present results demonstrate the progressive increase in the use of stored carbohydrate fuel within muscle during exercise of increasing intensity. The supply of circulating fuel (as glucose and FFA) also increases exponentially with work load but the utilisation of circulating fuel is more properly related to the duration of exercise. During the post-exercise period the removal excess mobilised FFA not used as fuel and not immediately re-esterified, is undertaken by liver, and blood ketone-body concentrations rise in proportion to the increases in plasma FFA.

1.5 SUMMARY

1. The relationship between exercise intensity and the metabolic changes in venous blood during and after exercise were examined in 5 subjects. The effect of increased duration of exercise was also studied in two of these subjects.

2. Blood samples were analysed for lactate, pyruvate, glucose, glycerol and free fatty acids. Heart rate was monitored by continuous e.c.g.

3. The increases in heart rate and in the metabolite concentrations

during exercise were positively correlated with exercise intensity. The correlation was improved in a logarithmic relationship.

4. The incremental areas of the change in metabolite concentrations were linearly related to the duration of exercise.

5. The results consider major differences in mobilisation and usage of stored fuels according to work load and work time. In particular the new observation has been made that the continued mobilisation of free fatty acids after exercise and the post-exercise rise in ketone-body concentrations is related to the intensity and duration of exercise.

CHAPTER 2

A COMPARISON OF THE EFFECTS OF STRENUOUS EXERCISE IN UNTRAINED SUBJECTS AND RACING CYCLISTS

2.1 INTRODUCTION

The investigations described in the previous chapter demonstrated that short term high intensity work resulted in a large flux of carbohydrate metabolites into the blood whereas longer-term moderate exercise resulted in a greater liberation of fat-derived substances. It would seem, therefore, that for any individual the utilisation of fat or carbohydrate for fuel would depend upon his relative work capacity.

In a comparison of well-trained athletes and untrained subjects who ran for 2 hr, a previous study showed differences in the metabolic response to exercise between the two groups. The trained runners had lower blood concentrations of lactate and pyruvate during exercise and lower concentrations of plasma free fatty acids (FFA) and blood ketone-bodies in the post-exercise period (Johnson, Walton, Krebs & Williamson, 1969; Johnson & Walton, 1972). These might be true metabolic differences between athletes and untrained subjects which could be innate, or due to adaptation as a result of athletic training. They could also be explained if the untrained subjects had worked relatively harder than the athletes by running to the limits of their capacity; the athletes, by running well within their limits, could have been subjected to smaller demands on their metabolic reserves. To investigate these possibilities we have studied a group of racing cyclists and also untrained subjects during and after a period of exercise in which the cyclists worked at greater loads and nearer to their maximal capacity. A preliminary report has been published (Jennett, Johnson &Rennie, 1972).

2.2 METHODS

2.2.1 Subjects

Four racing cyclists (males aged 23-31 yr) agreed to take part in the study. They had been training for at least 30 weeks by cycling about 200 miles per week and frequently competed in local and national events. The cyclists had a mean height of 175 cm (171-178) and a mean weight of 70 kg (63-82). Five control subjects (males aged 24-32 yr) were also studied. They did not take part in regular athletic training and had a mean height of 181 cm (172-189) and a mean weight of 83 kg (68-97).

2.2.2 Techniques

(a) Heartrate: Miniature chest electrodes (Grass) were applied. The e.c.g. was displayed on a cathode-ray oscilloscope (Tektronix 502), which triggered an instantaneous ratemeter (Devices Ltd.) and heart rate was recorded on one channel of an ultraviolet recorder (SEL).

(b) Ventilation and gas exchange: The subjects breathed through a rubber mouth piece attached to a low resistance Perspex valve with 500ml dead space (Zentral Werkstatt, Gottingen). The flow head for a pneumotachograph (Computing Electrospirometer type CS1, Mercury Electronics (Scotland) Ltd.) was placed on the inspiratory side of the valve. Expired air was passed through a tube of 100ml into a 6 litre mixing chamber, from the far end of which gas was continuously sampled through an infra-red CO_2 analyser (URAS, Hartmann & Braun) and a paramagnetic O_2 analyser (type 101, Servomex Controls Ltd.) connected in series. Gas analysers were calibrated immediately before each study with mixtures analysed using a Lloyd-Haldane

apparatus. Cumulative inspired volume and mixed expired O_2 and CO_2 concentrations were recorded continuously on the ultraviolet recorder. The pneumotachograph was calibrated for known flow rates using rotameters. With a setting to give a correct reading for flow between 100 and 200 l/min, there was a slight non-linearity at the lower and upper ends of the range (50, 300), within 3% of a correct reading. At the flow rates reached during exercise, this give a possible error of not more than 1% in the estimation of ventilation and, therefore, also of oxygen consumption. Inspired volume per minute was read from the chart record and corrected to give expired volume at STPD. The mean values for O_2 and CO_2 concentration in the expired air were taken from the record and O_2 consumption, CO_2 output and respiratory exchange ratio (R) were calculated using Dills nomogram (Quoted in Consalazio, Johnson & Pecora, 1963). Values were calculated from data averaged over periods of at least 5 min at rest and 2-3 min during exercise.

2.2.3 Procedure

The investigation was explained to the subjects and their consent obtained. They were studied in the morning after overnight fasting. A catheter was placed in the antecubital vein, fitted with a tap and a first resting blood sample was taken. It was flushed with saline (0.9%) and strapped to the forearm. Observations were made of ventilation, expired gas concentrations and heart rate until steady results were obtained for at least 5 min. A furtherresting blood sample was taken. The subjects then cycled for 20 min on a bicycle

ergometer (Monark). Cycling was started at a load and pedalling rate expected to raise the heart rate to 150-170/min. Some trial and error adjustment of the load was necessary but the required heart rate was attained during the first 5 min and all were within or above this range in the second 10 min. The mean and range of work loads maintained in the final 10 min were 1070 (1000-1160) kpm/min for the cyclists and 715 (630-866) kpm/min for the non-cyclists. Recording of ventilation, expired gas concentrations and heart rate was continued throughout the exercise period and for 10-15 min thereafter. Further recordings were made at 20 to 30 min (5 subjects) and at 50 to 60 min (all subjects) after the end of exercise. Venous blood samples were taken via the indwelling catheter at 5 min intervals during exercise and afterwards at 15-30 min intervals for 3 hr. Blood was analysed as described in Appendix 1 for metabolites, insulin and growth hormone. Significance of difference was examined with the Mann-Whitney u test.

2.3 RESULTS

(a) Heart rate: (Fig. 2.i) The cyclists attained higher heart rates but the difference between the group means attained significance ($P < 0.01$) for only the 10 min measurement. There was no significant difference in the recovery rate between the two groups as shown by the heart rates at 5 and 10 min after the end of exercise. At one hour afterwards heart rates were within 10 heats per min of pre-exercise values, with one exception in the untrained group.

(b) Ventilation: (Fig. 2.i) Ventilation was significantly higher in the cyclists before exercise but not after recovery

Fig. 2:i Heart rate (beats/min) and oxygen consumption (ml/min per kg body wt) in 4 racing cyclists (⊙ —— ⊙) and 5 untrained subjects (o ---- o) during and after 20 min of exercise (means ± SEM).

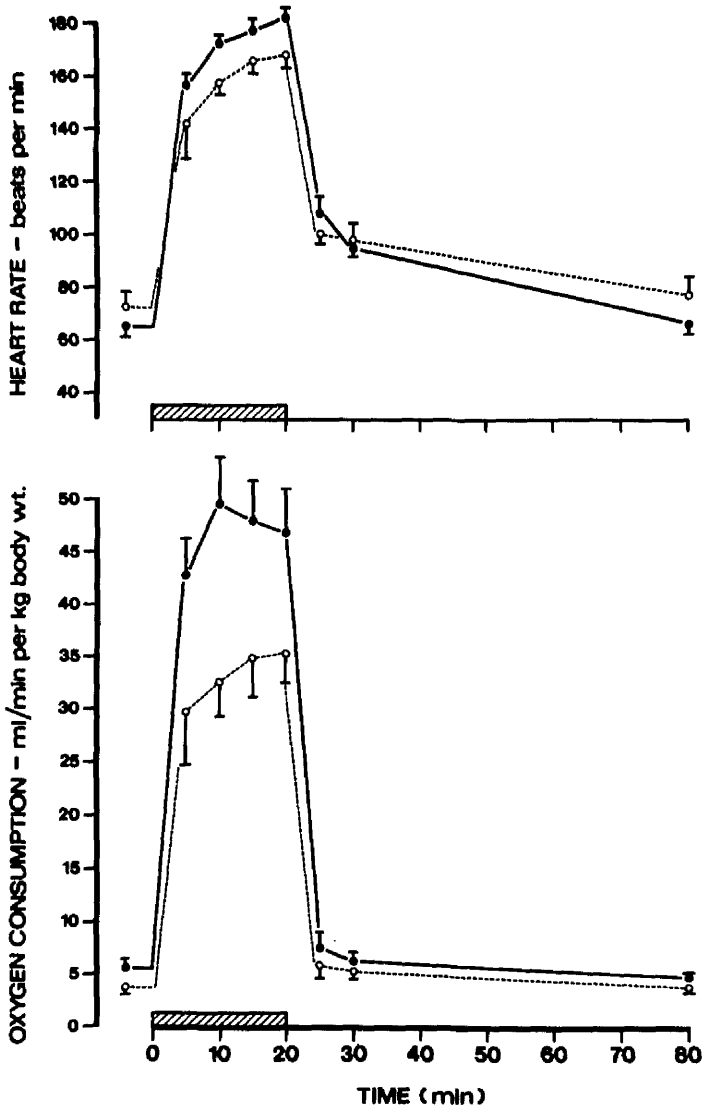
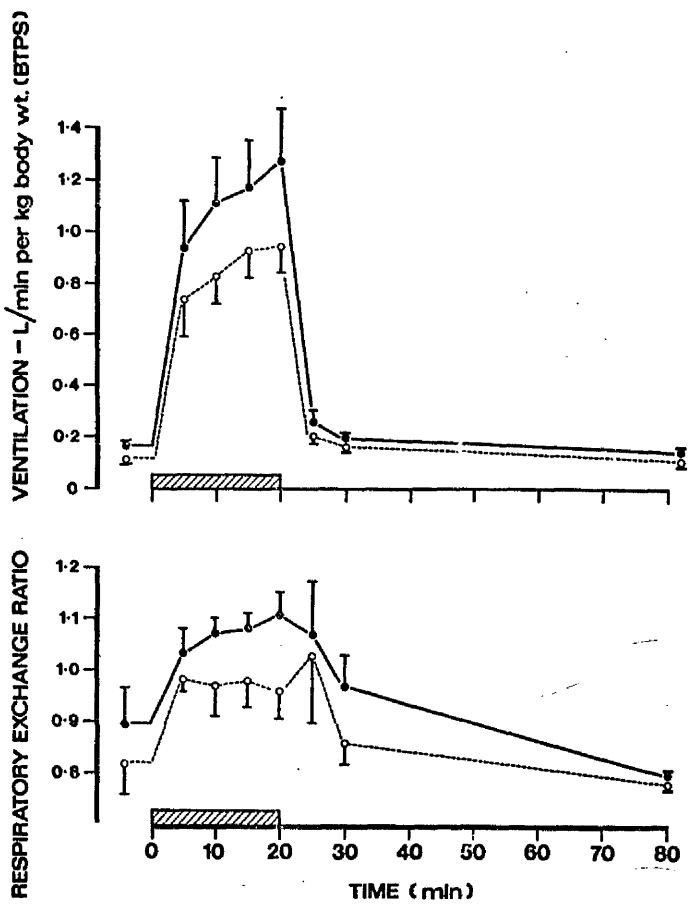

HEART RATE – beats per min
180
160
140
120
100
80
60
40
OXYGEN CONSUMPTION – ml/min per kg body wt.
50
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
80
TIME (min)

Fig. 2:ii Ventilation (l/min per kg body wt B.T.P.S.) and respiratory exchange ratio in 4 racing cyclists (⊙ —— ⊙) and 5 untrained subjects (o ---- o) during and after 20 min of exercise (means $\pm$ SEM).


VENTILATION – L/min per kg body wt. (BTPS)
1·4
1·2
1·0
0·8
0·6
0·4
0·2
0
RESPIRATORY EXCHANGE RATIO
1·2
1·1
1·0
0·9
0·8
0
10
20
30
40
50
60
70
80
TIME (min)

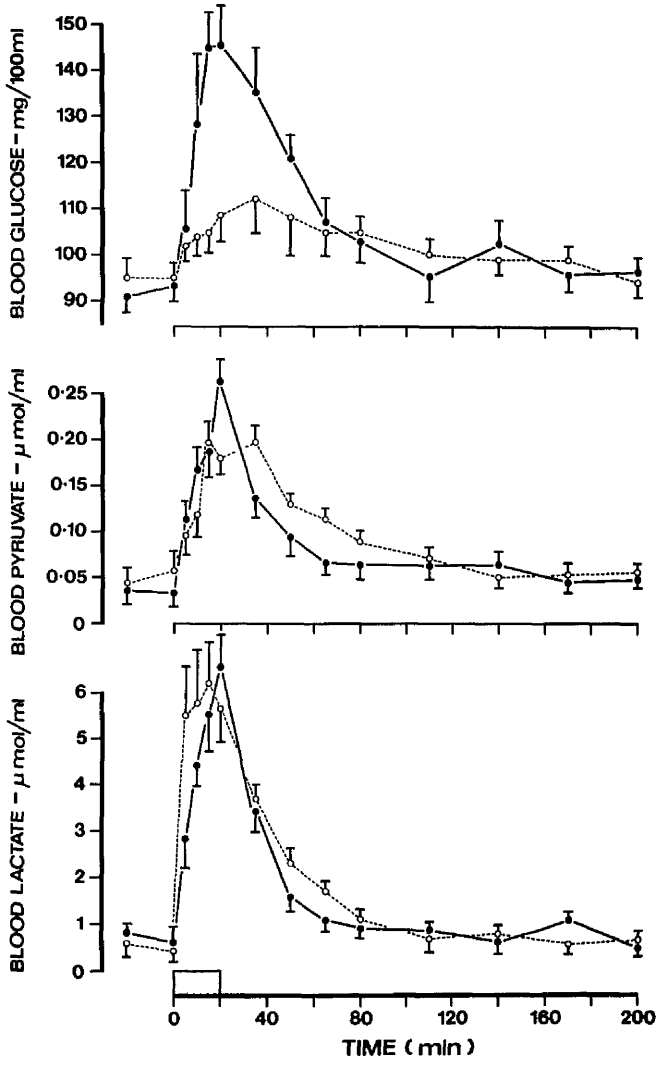
suggesting that they tended to over-ventilate in anticipation; this was confirmed by their higher R before exercise. Ventilation during exercise was higher for the cyclists but the difference did not reach significance. Maximal values for the cyclists ranged from 66-115 1/min (B.T.P.S.) and for the non-cyclists from 68-99 1/min. The values in the figures have been expressed at 1/min per kg body weight.

(c) Gas exchange: (Fig. 2.ii) The cyclists showed higher O_2 consumptions and the difference was significant at 10 and 15 min during exercise ($P < 0.01$) for mean values standardised to ml/min per kg body weight. Before standardisation for each of the cyclists the highest volume of O_2 consumption ranged from 2.5-3.7 1/min and from 2.4-3.2 1/min for the non-cyclists. Higher values of R tended to be attained by the cyclists but the differences did not reach significance.

(d) Pyruvate: (Fig. 2.iii) Resting concentrations of pyruvate were not significantly different in the two groups. Exercise caused increases in pyruvate concentrations in both groups, except that the cyclists had a significantly higher blood concentration at the end of 20 min of exercise ($P < 0.01$). In the cyclists pyruvate fell rapidly to concentrations significantly below the concentrations in the non-cyclists at 45 min after exercise ($P < 0.01$). The blood pyruvate in the untrained group did not fall to this concentration until 90 min after exercise.

(e) Lactate: (Fig. 2.iii) In both groups resting concentrations of lactate were the same but exercise caused

Fig. 2:iii Blood glucose (mg/100 ml), pyruvate (μmol/ml) and lactate (μmol/ml) in 4 racing cyclists (⊙ — ⊙) and 5 untrained subjects (o --- o) during and after 20 min of exercise (means ± SEM).

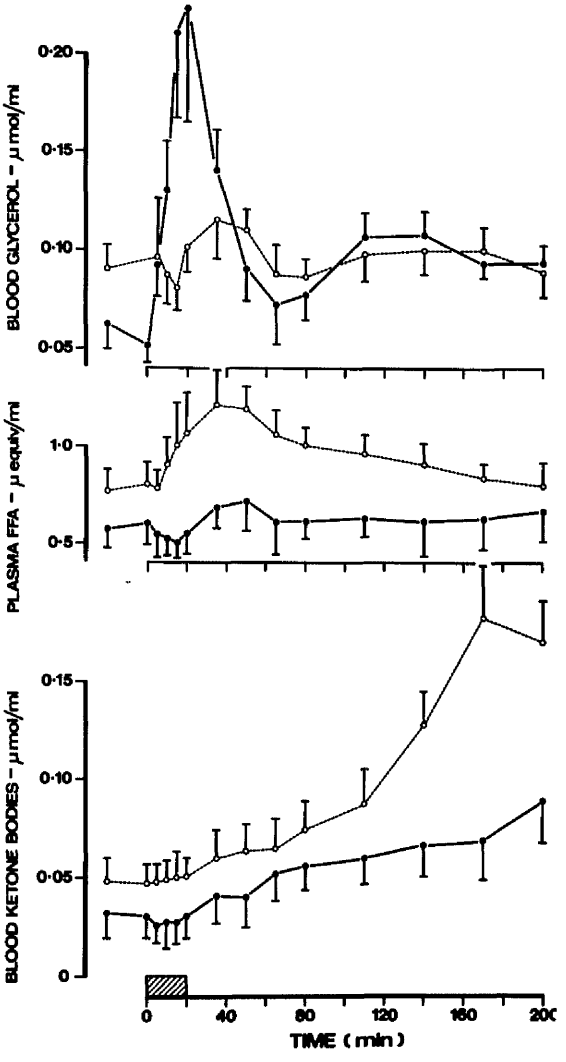

BLOOD GLUCOSE - mg/100ml
150
140
130
120
110
100
90
BLOOD PYRUVATE - μ mol/ml
0·25
0·20
0·15
0·10
0·05
0
BLOOD LACTATE - μ mol/ml
6
5
4
3
2
1
0
0
40
80
120
160
200
TIME (min)

a large (six-fold) increase of blood lactate above resting concentrations. The untrained subjects showed a much faster rise of blood lactate than the cyclists in the first 10 min of exercise but both groups reached similar concentrations at the end of the exercise period. The rate of return to resting concentrations in the post-exercise period was similar in the cyclists and untrained subjects.

(f) Glucose: (Fig. 2:iii) At rest, concentrations of blood glucose were similar in both groups. 20 min of exercise caused a rise in glucose concentrations by about 20% from resting levels in non-cyclists but the cyclists showed a greater rise (60%) above resting levels. A further small rise was seen in the control group 15 min after exercise, after which glucose concentrations fell slowly to resting values by 180 min after exercise. Blood glucose concentration in the cyclists fell rapidly after exercise to within 15 mg/100 ml of resting levels at 180 min after exercise. Glucose concentrations were significantly different in the two groups at 10, 15 and 20 min of exercise and at 15 min post-exercise ($P < 0.01$).

(g) Glycerol: (Fig. 2:iv) The cyclists had significantly lower concentrations of blood glycerol at rest than the untrained group ($P < 0.05$). During exercise concentrations of glycerol rose rapidly in the cyclists but were not significantly altered in the non-cyclists. The difference was highly significant. In the first 45 min after exercise, the glycerol concentration fell rapidly from its high value in the cyclists whilst in the

Fig. 2:iv Blood glycerol (μmol/ml), plasma FFA (μequiv/ml) and blood ketone-bodies (μmol/ml) in 4 racing cyclists (● —— ●) and 5 untrained subjects (○ ---- ○) during and after 20 min of exercise (means ± SEM).


BLOOD GLYCEROL – µ mol/ml
0·20
0·15
0·10
0·05
PLASMA FFA – µ equiv/ml
1·0
0·5
BLOOD KETONE BODIES – µ mol/ml
0·15
0·10
0·05
0
0
40
80
120
160
200
TIME (min)

untrained group the concentration rose slightly. The glycerol concentrations in the untrained group approximated to their resting values within 50 min of the end of exercise but in the cyclists the concentrations were significantly raised above their resting values for the remainder of the investigation (3 hr).

(h) Plasma FFA: (Fig. 2:iv) FFA concentrations were significantly lower before exercise in the cyclists than in the untrained subjects ($P < 0.01$). During exercise in both groups FFA first fell and then rose. In the untrained group the fall was slight and was observed only at 5 min. In the cyclists it was greater and persisted for 15 min. In both groups FFA concentrations then rose in the remaining part of exercise and for 30 min afterwards but this rise was greater for the untrained group (to 150% of resting) than the cyclists (to 130% of resting). In the remaining period of the investigation concentrations of FFA slowly returned to within 10% of their resting values in both groups.

(i) Ketone-bodies: (Fig. 2:iv & 2:v) Resting concentrations of total blood ketone-bodies (acetoacetate plus 3-hydroxybutyrate) were slightly higher in the untrained group compared with the cyclists. In neither group was there a significant change during exercise but in both groups concentrations of ketone-bodies rose in the post-exercise period. The rise was most marked for the untrained group in whom the increase was about 350% of the resting concentrations by 150 min after exercise. The rise in ketone-bodies for the cyclists over the same period was 200%. In the remaining 30 min of the investigation the ketone-body concentrations fell slightly in the non-cyclists and rose slightly in the cyclists.

Fig. 2:v Relationship between exercise $\dot{V}O_2$ and (a) blood concentration of ketone-bodies 150 min after exercise, (b) heart rate during exercise. (Individual values; ⊙ : racing cyclists; o : untrained subjects).

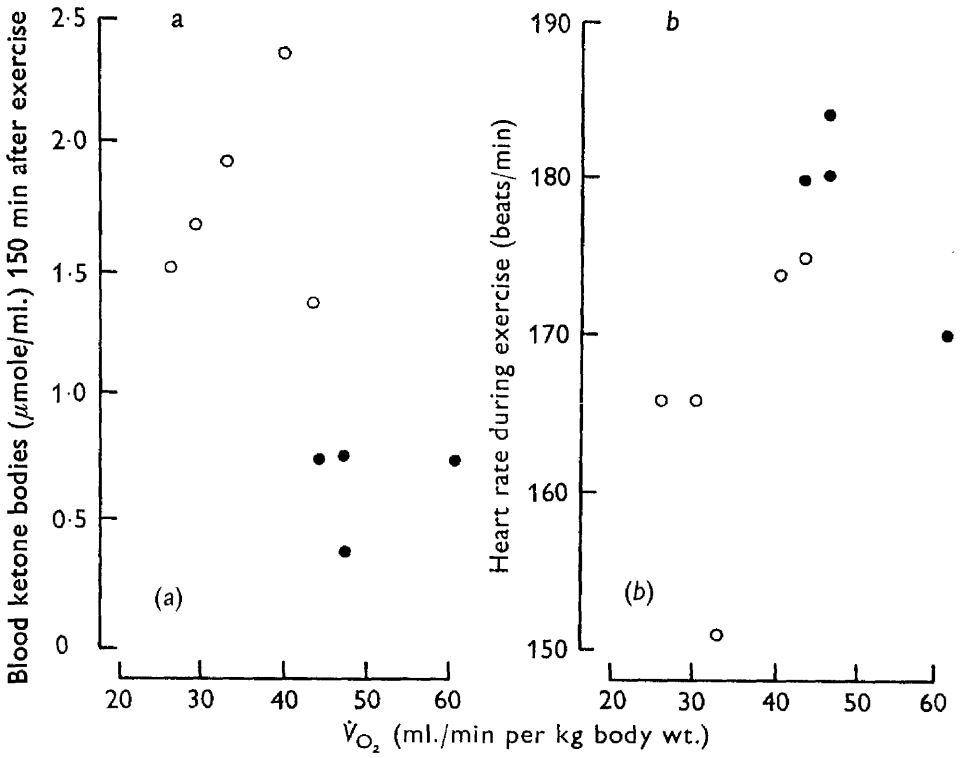
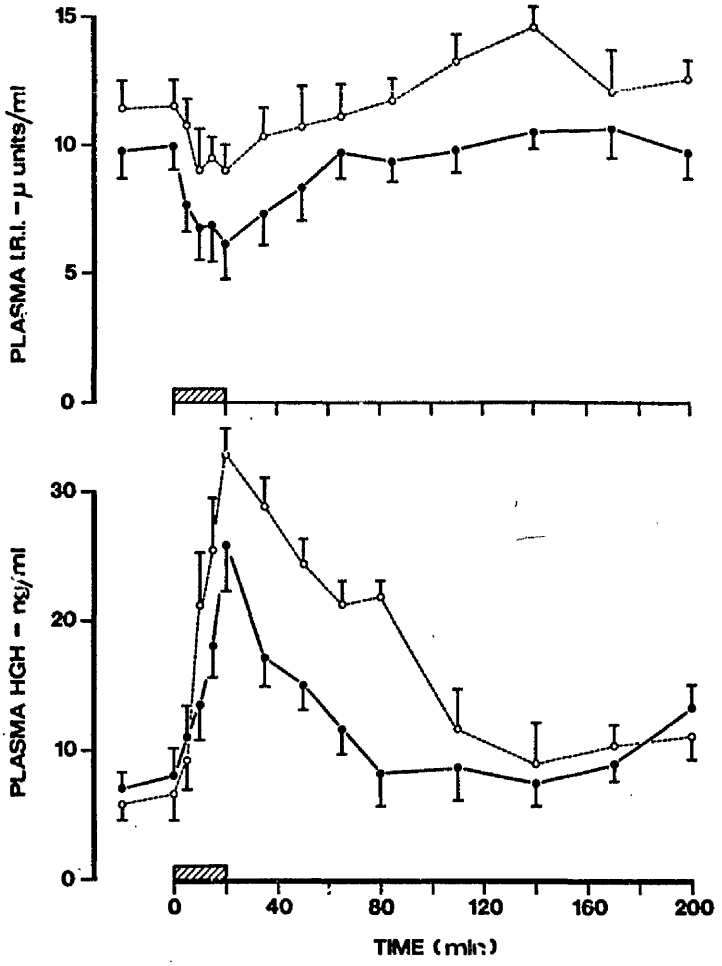

Blood ketone bodies (μmole/ml.) 150 min after exercise
2·5
2·0
1·5
1·0
0·5
0
a
(a)
20
30
40
50
60
Heart rate during exercise (beats/min)
190
180
170
160
150
b
(b)
20
30
40
50
60
$\dot{V}_{O_2}$ (ml./min per kg body wt.)

Fig. 2:vi Plasma immunoreactive insulin (μ units/ml) and plasma human growth hormone (ng/ml) in 4 cyclists (o — o) and 5 untrained subjects (o - - - o) during and after 20 min of exercise (means ± SEM).


15
10
5
0
PLASMA I.R.I. – μ units/ml
30
20
10
0
PLASMA HGH – ng/ml
0
40
80
120
160
200
TIME (min)

(j) Insulin: (2:vi) The cyclists had slightly lower resting concentrations of plasma IRI than the non-cyclists but the differences were not significant. Exercise caused a fall in values of IRI concentration in both groups but the fall by the end of exercise was significantly greater ($P < 0.05$) in the cyclists (40%) from that in the non-cyclists (25%). In both groups plasma IRI concentrations had returned to resting values by 80 min after exercise.

(k) Growth Hormone: (Fig. 2:vi) Resting concentrations of HGH were similar in the cyclists and non-cyclists. In both groups exercise increased the values of HGH but the increase was larger in the non-cyclists so that at the end of exercise the difference between the two groups was highly significant ($P < 0.01$).

2.4 DISCUSSION

Earlier comparisons of trained and untrained runners, in which the trained subjects ran faster but with lower heart rates, showed that the athletes had much smaller increases in blood concentrations of lactate and pyruvate during exercise and a lesser degree of post-exercise ketosis (Johnson et al., 1969). One of the purposes of the present investigation was to exclude the possibility that the earlier findings of greater post-exercise ketosis in untrained subjects might have been related to a greater relative work load. The investigation was therefore designed so that the cyclists should work as hard if not harder than the untrained subjects. Within the limitation of a single session per subject the exercising heart rate was taken to be the best guide to their relative work output (% VO_2 max) since

heart rate is similar in subjects of the same age and sex working at the same percentage of maximal aerobic capacity (Åstrand, Cuddy, Saltin & Stenberg, 1964). The load was, therefore, adjusted in the first few minutes to cause a similar increase in heart rate in all subjects. In practice the cyclists showed a tendency to work harder than required and, on average, reached higher heart rates. None of the other measurements suggested that the cyclists attained a lower percentage of their maximal aerobic capacity: indeed there was evidence that they worked relatively harder. Therefore the differences between the two groups cannot be due to the untrained subjects working harder.

The cyclists had a slightly greater rate of increase of pyruvate at the beginning of exercise and also a slower rate of lactate increase compared with controls. As a result the lactate-pyruvate ratio in the cyclists was lower, which would be expected under conditions of increased glycolysis in muscles of higher oxidative capacity. A relative increase in the oxidative capacity of muscle mitochondria is likely to be more important than a simple increase of muscle tissue as investigations in animals have shown that training causes this effect (Holloszy, 1967; Oscai & Holloszy, 1971). It is unlikely that lactate and pyruvate production by muscle were related to low tissue oxygen tensions because heavy exercise does not cause oxygen tensions low enough to depress oxidative phosphorylation (Chance, Shoener & Schindler, 1964). The rate of oxidation of pyruvate is probably more dependent upon the substrate handling capacity of the pyruvate-decarboxylase system than upon the supply of oxygen to the cell (Keul, Doll, Erichsen & Reindell, 1968; Molé, Baldwin, Terjung & Holloszy, 1973).

Oxygen consumption after exercise fell more quickly than the blood concentrations of lactate and pyruvate indicating that O_2 debt and the concentrations of these metabolites are not directly related (Harris, 1969).

There were marked differences between the two groups in the effects of exercise on the blood concentrations of other metabolites. The much greater rise in glucose concentration in the cyclists was remarkable. The large increase in blood glucose concentration in the cyclists was probably related to a decrease in the uptake of glucose by muscle since plasma IRI fell to a greater extent in the cyclists and circulating glucose is not a major fuel for working muscle until muscle glycogen concentrations fall (Hermansen, Pruett, Osnes & Giere, 1970).

Working muscle is able to oxidise FFA; ketone-bodies produced as a result of the oxidation of FFA in the liver are also used as a fuel. The large and rapid rise of glycerol concentration during exercise in the cyclists suggests that they mobilised more fat than the non-cyclists, suggesting that they utilised FFA liberated during exercise more efficiently. The oxidation of FFA and ketone-bodies in muscle, even when glycolysis occurred, would be advantageous, reducing equivalents from FFA could be coupled via the flavoprotein-NAD system to oxidative phosphorylation. This would result in production of ATP without involving the pyruvate-decarboxylase system. As the concentration of blood ketone-bodies hardly altered during exercise there was probably an equilibrium between production and utilisation. After exercise, however, when muscle utilisation of fuel ceased, there was a rise in the ketone-body

concentration. This was unrelated in either its magnitude or timing to the oxygen debt. The much greater rise of ketone-bodies after exercise in the untrained subjects despite the lower levels of lipolysis probably reflected their lower utilisation and continued breakdown of FFA by the liver (Johnson & Walton, 1972). It is likely that the disappearance of fat metabolites from the blood of the cyclists would be faster after exercise because increased glucose utilisation suggested by the rapid fall of glucose concentrations promotes re-esterification and lipogenesis in peripheral adipose tissue.

The values of gas exchange ratio (R) were high particularly for the cyclists and are difficult to reconcile with the present suggestion that a higher proportion of fat was utilised by these subjects. It is unlikely that any consistent error of gas analysis would account for the high values; analyses were calculated frequently and with various standard mixtures; also the resting values for VO_2, VCO_2 and R, derived by the same methods throughout, were appropriate before and after the exercise periods. A high value for R may be partly due to a changing acid-base state when blood lactate is rising and bicarbonate falling (Wasserman, van Kessel & Burton, 1967). The trained subjects might have increased their ventilation more efficiently in response to decreasing blood pH and might, therefore, tend to have high R values. A higher R might also occur due to relative differences in substrate utilisation by this group. For example, the athletes may use a greater proportion of ketone-bodies as a fuel in muscle (Winder, Baldwin & Holloszy, 1973) and their R is closer to 1 than that of fat.

A fall in the level of plasma IRI with exercise has been observed previously in normal subjects (Cochran, Marbach, Poucher, Steinberg & Gwinup, 1966). Lower concentrations of plasma IRI have been reported after a training programme (Devlin, 1963; Bjorntorp, De Jange, Sjöström & Sullivan, 1970; Bjorntorp, Fahlen, Grimby, Gustafson, Holm, Renstrom & Schersten, 1972) and the present observations confirm their findings. The greater fall during exercise of plasma IRI observed in the cyclists could be related to a greater relative work output but this requires further study.

A smaller increase in HGH concentration with exercise in trained athletes compared to untrained subjects has been previously reported (Sutton, Young, Lazarus, Hickie & Maksvytis, 1968; Sutton, Young, Lazarus, Hickie & Maksvytis, 1969). Nevertheless, HGH secretion does not seem to be important in the development of post-exercise ketosis since this occurs in patients with hypopituitarism (Johnson, Rennie, Walton & Webster, 1971). The lower values of HGH concentration in the cyclists compared to the untrained subjects were probably not related to differences in catecholamine secretion as an increase in circulating catecholamines does not affect HGH secretion in exercise (Troyer, Friedberg, Horton & Bogdonoff, 1966; Blackard & Hubbell, 1970).

Although previous observations suggested a fundamental difference in metabolism of ketone-bodies between athletes and non-athletes (Johnson et al., 1969; Johnson & Walton, 1972), the possibility existed that the metabolic differences were related to different relative work loads. The present observations confirm the previous results and indicate that the differences remained even when the athletically fit individuals worked at least as hard or harder.

2.5 SUMMARY

1. In order to investigate the relationship of the degree of post-exercise ketosis to athletic fitness four racing cyclists and five untrained subjects were studied during and after 20 min strenuous exercise on a bicycle ergometer. The work loads were adjusted so that the cyclists worked nearer their maximal capacity than the untrained subjects. Observations were made of respiratory gas exchange and blood samples were taken at rest during and after the exercise for subsequent analysis for metabolites, insulin and growth hormone.

2. The cyclists worked at 1070 kpm/min with heart rate of 179.5 beats/min and oxygen consumption of 48 ml/min per kg body weight (means). The values for the untrained subjects were 715, 167 and 35 respectively.

3. During exercise the concentrations of glucose and glycerol in the blood rose higher in the cyclists compared with the untrained subjects. The concentrations of plasma free fatty acids were, however, lower.

4. After exercise the concentration of ketone-bodies in the blood increased progressively in both groups, reaching highest measured values 150 min after exercise. The increase was much smaller for the cyclists even though they worked harder.

5. It is concluded that the results indicate that the extent of post-exercise ketosis reflects true metabolic differences associated with athletic training.

CHAPTER 3

THE ALTERATION BY A TRAINING PROGRAMME OF THE METABOLIC AND HORMONAL RESPONSE TO RUNNING

3.1 INTRODUCTION

Results in the preceding chapter demonstrate metabolic differences in the response of trained athletes and untrained controls to exercise upon a bicycle. These differences are thought to represent the effects of athletic training and should, therefore, be observed in normal individuals who undergo a period of physical training. Should the differences at present associated with athletic training not be seen after a training programme then some other explanation would be needed, perhaps on the basis of the innate difference of persons who become athletes, compared to those persons who do not.

3.2 PROCEDURE

Eight teachers, young men who had recently gained their teaching certificate, were studied during an additional course they took to specialise in the teaching of games. Although all the subjects played team games and were interested in physical culture none of them was described by their supervisor as being athletically fit and none had ever run in long distance races. They were not in regular athletic training although they had fitness-indices considerably above average.

The investigation, in outline, was as follows: the subjects should run for a period of two hours during and after which blood samples would be taken. Then they should undergo a period of training before repeating the run at a later date. The investigation was explained to the subjects and they agreed to take part. They were to train by running for 30 min three times a week for three weeks and the investigation would be completed by studying them again on the first day of the fourth week.

The runners presented themselves, after fasting for 15 hr, at 0900 hr on the morning of the first run. They were weighed and their heights measured. They reported the results of the Harvard step-test (Brouha, 1943) which had been carried out on the previous day. Resting blood samples were taken by venepuncture and the subjects set off on the run. They returned after 30 min when a second sample was taken by venepuncture and they set off again. After running for a further 30 min they had venous catheters inserted and the remaining blood samples were taken through these at the end of the run and for 90 min afterwards. Blood samples were kept on ice until they were analysed for metabolites and hormones as described in Appendix I. The entire procedure was repeated after the training period.

3.3 RESULTS

3.3.1 ATHLETIC FITNESS

The runners showed marked improvement in their fitness judged from their performance in the Harvard step-test (see Table 3:ii). They ran the same distance on the second occasion as on the first and they took the same time but reported that it had seemed easier after the training.

3.3.2 METABOLITE CHANGES

(a) Blood glucose: (Fig. 3:i) The period of training did not alter the resting levels of glucose. However, the rise in blood glucose concentration during the run was greater afterwards and the levels during the post-training run were significantly elevated ($P < 0.01$ paired Student's t test).

Table 3:i Details of subjects who underwent training for three weeks between investigation of the metabolic changes during and after running for 1 hr.

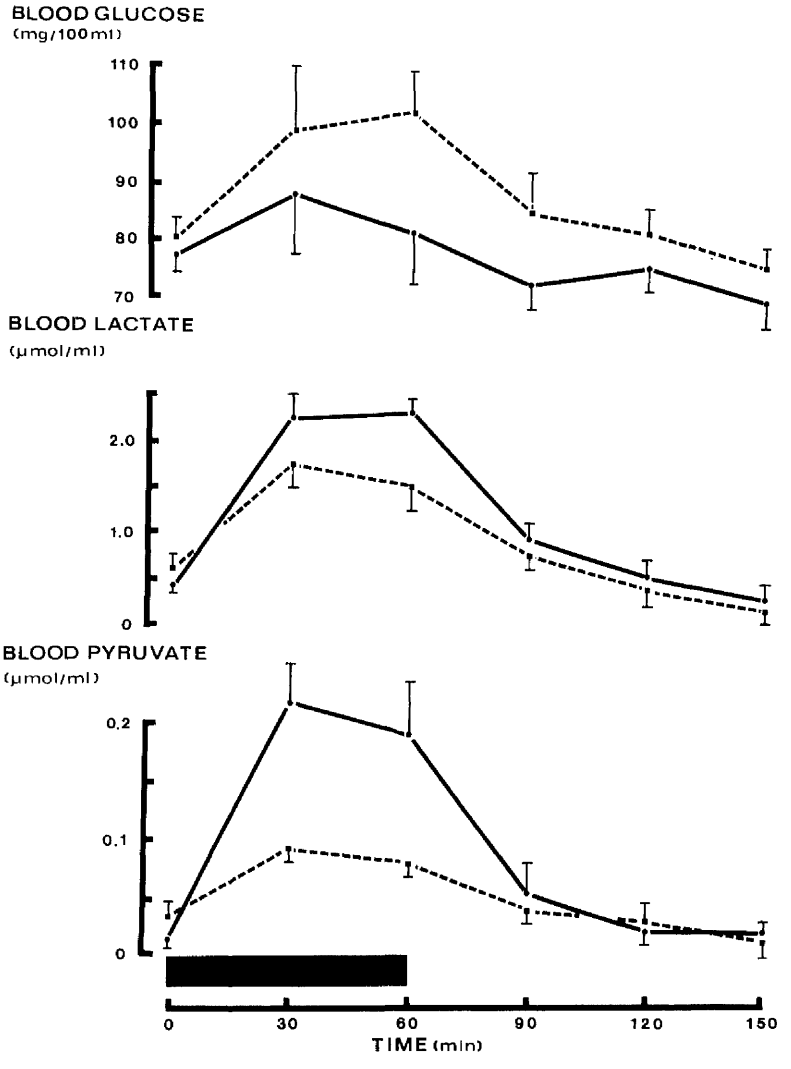
Subject	Age (yr)	Height (cm)	Weight (kg)
D.P.	21	190	80.3
V.K.	29	178	68.0
D.F.	23	188	88.9
D.C.	23	180	69.4
C.T.	23	183	70.3
P.McM.	24	180	76.2
M.S.	23	180	83.9
P.M.	24	186	83.9
Mean	23.75	183.1	76.6
± SEM	0.81	1.6	2.6

Table 3:ii Harvard Fitness Index (HFI) before and after training.

Subject	HFI Before	HFI After	Change
D.P.	99	108	+9
V.K.	106	124	+18
D.F.	100	107	+7
D.C.	93	109	+16
C.T.	98	118	+20
P.McM.	81	84	+3
M.S.	90	99	+9
P.M.	104	127	+23
Mean	96.5	110.0	
± SEM	2.85	3.73	

Significance of difference by paired Student's t test $P < 0.01$.

Fig. 3:i Changes during and after 1 hr running in blood glucose (mg/100 ml), lactate (μmol/ml) and pyruvate (μmol/ml) in 8 subjects before (● — ●) and after (o ---- o) physical training (means ± SEM)


BLOOD GLUCOSE
(mg/100ml)
110
100
90
80
70
BLOOD LACTATE
(μmol/ml)
2.0
1.0
0
BLOOD PYRUVATE
(μmol/ml)
0.2
0.1
0
0
30
60
90
120
150
TIME (min)

(b) Blood lactate and pyruvate: (Fig.3:i; Table 3:iii)

Lactate and pyruvate concentrations in the blood at rest were greater after the period of training. The change was significant ($P < 0.05$). During the run, however, the subjects had significantly lower levels of lactate and pyruvate after the period of training ($P < 0.001$). Lactate and pyruvate was also lower 30 min after the second run and lactate remained lower for the period of the remainder of the investigation. The lactate-pyruvate ratio was significantly lower at rest but fell less during exercise after training. (see Table 3:iii)

(c) Blood glycerol: (Fig. 3:ii)

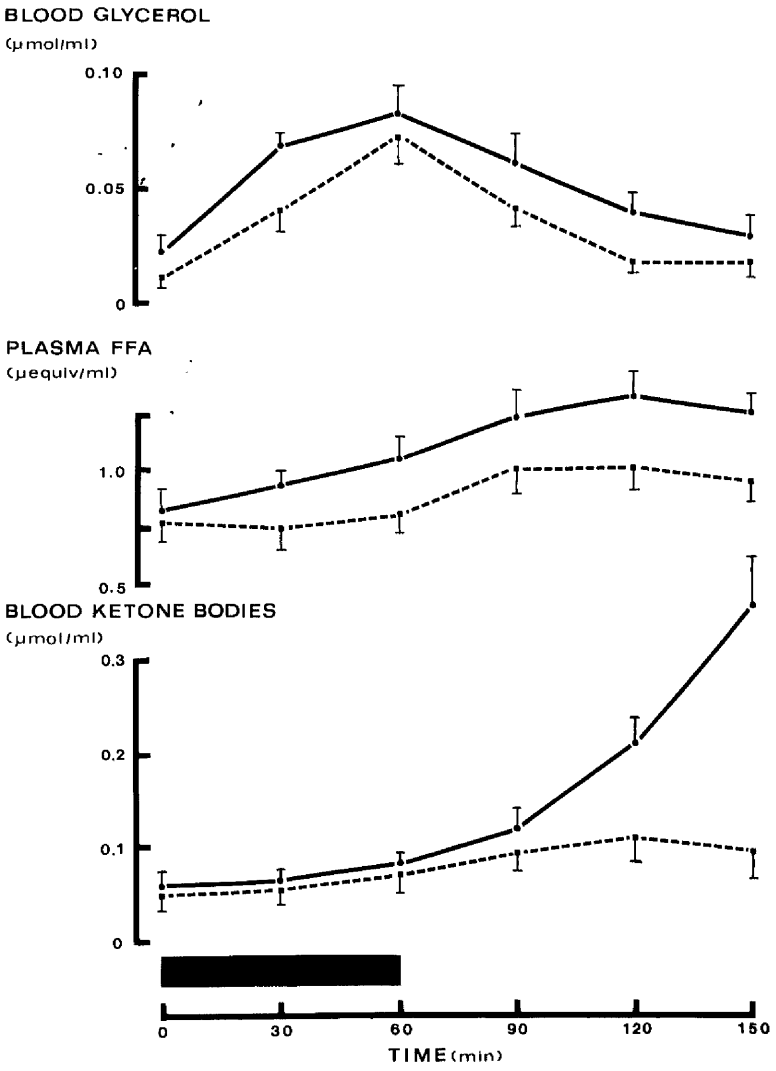
Blood glycerol concentrations were much lower at rest after the period of training (0.0215 ± 0.007; 0.009 ± 0.005 μ mol/ml). The levels of glycerol remained lower throughout the period of the second investigation although the percentage change in the concentration during exercise was greater (30 min % of resting level, before 186%, after 358%).

(d) Plasma FFA: (Fig. 3:ii) There was no difference in the resting level of FFA before and after training. However, the plasma concentrations were lower throughout the period of the second investigation. There was a significant ($P < 0.05$) fall in the plasma level of FFA at 30 min of running after training but not before. On both occasions FFA levels were elevated at the end of the run compared to the resting values and they continued to rise in the first 60 min after exercise. The greatest difference in the plasma FFA concentration, however, was later, 90 min after the end of exercise (1.310 ± 0.118, 1.020 ± 0.090 μ equiv/ml; $P < 0.001$).

Table 3:iii Changes with exercise and afterwards in lactate-pyruvate ratio (L/P) and 3-hydroxybutyrate-acetoacetate ratio (3HB/AcAc) before and after training.

		Running			Post - Exercise		
		Rest	30 min	60 min	+30 min	+60 min	+90 min
L/P	Before training	32.7	10.7	8.9	17.5	28.5	14.6
	After training	21.6	19.0	19.0	18.5	17.8	11.8
3HB/AcAc	Before training	2.94	2.94	4.78	3.50	3.94	3.13
	After training	2.96	4.20	4.18	2.81	2.87	2.90

Fig. 3:ii Changes during and after 1 hr running in blood glycerol (μmol/ml), plasma FFA (μequiv/ml) and blood ketone-bodies (μmol/ml) in 8 subjects before (● — ●) and after (o ---- o) physical training (means ± SEM).


BLOOD GLYCEROL
(μmol/ml)
0.10
0.05
0
PLASMA FFA
(μequiv/ml)
1.0
0.5
BLOOD KETONE BODIES
(μmol/ml)
0.3
0.2
0.1
0
0
30
60
90
120
150
TIME (min)

(e) Blood ketone-bodies: (Fig. 3:ii; Table 3:iii)

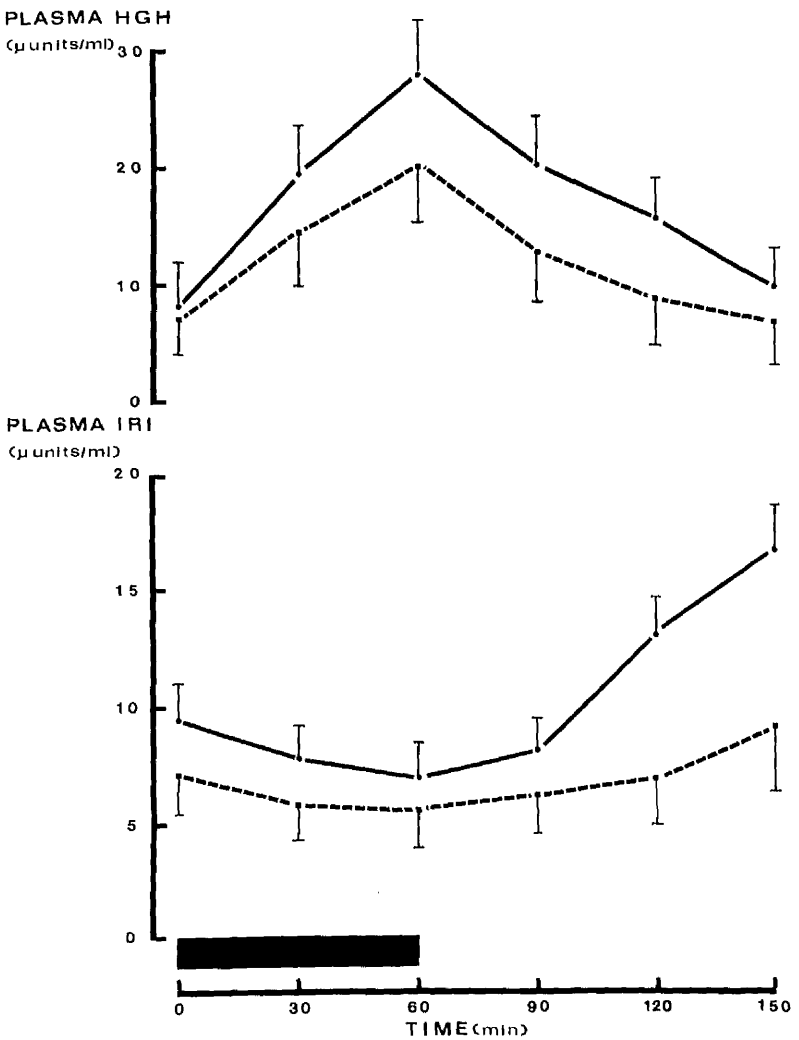
There was no change in the resting level of total ketone-bodies (acetoacetate plus 3-hydroxybutyrate) after training. There was a significant increase in the concentration of ketone-bodies after 30 min running on the second occasion and the 3HB/AcAc ratio was much higher after training at this time (2.92 before; 4.20 after). However, the value of the blood concentrations were lower after training at the end of exercise and throughout the post-exercise period. The greatest difference was at 90 min after the end of exercise when the values before training were almost four times those afterwards. The ratio (3HB/AcAc) of the ketone-bodies after exercise was significantly greater before training (Table 3:iii).

3.3.3 HORMONE CHANGES (Fig. 3:iii)

(a) Plasma IRI: There was a significant fall in resting levels of insulin after training ($P < 0.05$). After training the plasma insulin concentration fell less markedly during the run (19.8% after, 26.4% before) and the increase in insulin levels after exercise was also less than before training. The post-exercise increase before training was 75% of the resting level but afterwards the rise was only 36% above resting.

(b) Plasma HGH: The plasma concentration of growth hormone was similar at rest on both occasions. There was a smaller increase during exercise in the post-training run and the differences were significant during the period of running and 30 and 60 min afterwards.

Fig. 3:iii Changes during and after 1 hr running in plasma HGH (μunits/ml) and plasma IRI (μunits/ml) before (● —— ●) and after (o --- o) physical training (means ± SEM).


PLASMA HGH
(μunits/ml)
30
20
10
0
PLASMA IRI
(μunits/ml)
20
15
10
5
0
0
30
60
90
120
150
TIME(min)

3.4 DISCUSSION

The subjects showed a marked increase in fitness as judged by the Harvard step-test. They also reported that after running on the second occasion they felt much less tired than after the pre-training run. There is little doubt that their performance as endurance-runners must have increased and they may have run at a lower relative percentage of their maximum work capacity on the second occasion.

The increase in blood glucose levels during endurance exercise at work loads greater than 20% of maximum has been previously reported (Pruett, 1970a; Wahren, Ahlborg, Felig & Jorfeldt, 1971) and was also observed in the present work on trained athletes and untrained controls in ergometer exercise (Chapters 2&4). The greater increase in blood glucose in trained persons has not been previously examined in detail. The greater blood levels of glucose after training might be the result of greater hepatic glucose production stimulated by catecholamines. It is likely that trained subjects secrete less catecholamines (see Chapter 4) than untrained subjects at similar relative work loads. There is, however, the possibility of increased sensitivity to catecholamines released from adrenergic nerve terminals. Wahren and co-workers (1971) have shown that glucose uptake by muscle in endurance exercise increases with arterial concentration. It seems, therefore, that the increase after training in blood glucose concentration during exercise primarily reflects decreases in glucose uptake by muscle as a result of increased muscle glycogenolysis . However, since the normal stimulation of insulin secretion by hyperglycaemia is apparently over-ridden during exercise probably by an increase in

circulating catecholamine levels (Pruett, 1970; Porte, 1967) then the mechanism of glucose uptake by contracting muscle must be altered. One possible explanation would be an increase in insulin sensitivity of muscle or changes in the insulin-binding activity of muscle (Björntorp, De Jange, Sjöstrom & Sullivan, 1970; Wahlqvist, Kaijser, Lassers, Löw & Carlson, 1972b).

The present observations of increased lactate and pyruvate concentration at rest after training are puzzling when compared to the decreased blood concentrations during and after exercise. The lactate-pyruvate ratio at rest fell after the training programme so it appears that the pyruvate dehydrogenase system for removal of lactate was not overloaded, a mechanism previously suggested for lactate build up (Keul et al., 1968). It is possible that the increase simply reflects a change in the permeability of muscle cell membranes, which are said to become more permeable to electrolytes in the early stages of athletic training (Highman & Altland, 1963; Nichols, Spence, Hazlewood, Librik, Sachen & Clayton, 1972). Certainly in racing cyclists previously examined after a full competetive season (Chapters 2 & 4) and in subjects examined by other workers after 7 months training, lactate and pyruvate levels were lower at rest than in untrained subjects (Saltin & Karlsson, 1971).

The smaller increases with exercise in lactate and pyruvate after training have been previously observed (Chapters 2 & 4). An adequate explanation for this phenomenon is not yet available but it is unlikely that trained subjects break down less glucose to pyruvate than untrained subjects during work at similar relative loads since the rate of glycogen depletion in muscle

is independent of training (Saltin & Karlsson, 1971). The removal of lactate for gluconeogenesis by liver is not likely to be important as splanchnic uptake of lactate is not increased during exercise in untrained subjects (Rowell, Kraning, Evans, Kennedy, Blackman & Kusumui, 1966). The most likely explanation until recently appeared to be the more efficient oxidation of pyruvate (and thus lactate) within the increased numbers of mitochondria observed in muscle of trained subjects (Holloszy, 1967; Kiessling, Pichl & Lundqvist, 1971). However, recent evidence suggests that pyruvate may be removed by an increased generation of alanine by glutamate-pyruvate transaminase (Molé et al.,1973). This pathway also provides α-ketoglutarate to replenish citric acid cycle intermediates which become depleted as oxalacetate is condensed with acetyl-CoA from FFA oxidation. The present results show no change during exercise after training. However, the ratio fell during exercise before training because of an increase in pyruvate. This suggests that the control of the ratio of lactate to pyruvate becomes more accurate in trained muscle and is not much changed by increased production during exercise. This adds weight to the theory of altered pathways of pyruvate removal after athletic training (Molé et al, 1973).

In chapter 2 it was suggested that trained individuals used a greater proportion of fat as fuel during exercise. Although FFA and glycerol levels were lower after training the present results support the suggestion of greater increase in lipolysis in terms of the FFA-glycerol ratio during exercise after training. However, since FFA is taken up by muscle according to blood concentrations it is difficult to reconcile the suggestion of

greater utilisation with the lower levels of plasma FFA after training unless the FFA are used more rapidly. Evidence for this comes from the decrease after training in plasma FFA after 30 min of running and the concommitant small increase in blood ketone-body concentration suggesting a rapid breakdown of FFA to acetyl sub-units. The percentage increase in blood glycerol was also greater after training, indicating a greater supply of FFA after lipolysis. Also, although plasma FFA levels had risen by 59% at the end of exercise before training the production of ketone-bodies resulted in an increase of 500% After training the figures are 22% and 110%, indicating a more efficient oxidative metabolism of FFA, since build up of ketones results from incomplete oxidation of acetyl residues in the Kreb's cycle. The more efficient breakdown of FFA is also emphasised by the low 3HB/AcAc ratio. This ratio tends to increase with increasing levels of ketone-bodies as mitochondrial equilibrium

$$AcAc + NADH \rightleftharpoons NAD + 3HB$$

is shifted to the right as the metabolism of acetoacetate via acetyl CoA slows down.

The present findings of lower levels of growth hormone during exercise after training are similar to previous results from a comparison of racing cyclists and untrained subjects (Chapters 2 & 4). Other workers have shown decreased HGH levels in a cross sectional study of trained and untrained subjects (Sutton, Young, Lazarus, Hickie & Maksvytis, 1968) but the effect of increased athletic fitness in the same subjects has not previously been shown. Evidence that the lower levels of HGH are probably not related to the lower plasma FFA

concentration and the absence of post-exercise ketosis is presented in Chapter 7.

The lower levels of plasma insulin at rest after athletic training have been previously observed in obese and middle-aged subjects (Björntorp et al., 1970,72) but there have been no previous reports of the lowering effect by training in a longitudinal study of young men. The mechanism of the lowering of the resting values is unknown and is not apparently related to resting blood glucose values. A decrease in insulin secretion or an increase in insulin uptake peripherally would both explain the lower plasma levels. Björntorp et al., have evidence of normal glucose tolerance tests in obese patients with insulin lowered after physical conditioning and suggest that peripheral insulin sensitivity is increased by training.

The decrease of plasma insulin during exercise, probably due to catecholamine suppression of insulin release, has previously been reported to be smaller after training (Devlin, 1963). In cross sectional studies of well-trained and untrained subjects Pruett (1970b) noted no differences. However, in the present longitudinal study such differences are more likely to be seen since the precision of analysis of different increases. There is no explanation readily available for the different levels of insulin after training. Since the subjects probably improved their exercise capacity during training it is possible that by running at a relatively lower proportion of their maximal work load capacity the catecholamine induced suppression of insulin release occurred at a lower rate (see Chapter 4).

The post-exercise changes in plasma insulin and in ketone-bodies support the suggestion made elsewhere in this thesis of stimulation of the pancreas by ketone-bodies to produce insulin (see Chapters 7 & 9).

The results presented in this chapter show that a programme of physical training by running produces a pattern of increased oxidative metabolism of carbohydrate and fat similar to that previously observed in cross sectional studies of athletes. There are also changes in insulin and growth hormone, which have not previously been observed, as a result of physical training in the same individuals. Although the changes may be related to an increase in work capacity during training the relationship is not direct, as shown best by the large apparent increase in oxidative FFA metabolism and thus decreased ketone-body concentrations after exercise.

3.5 SUMMARY

1. Eight young men underwent a programme of training by running for 30 min at moderate speed three times a week for three weeks.

2. Metabolic and hormonal changes in blood were studied during and after a run of 60 min at comparable speeds before and at the end of the training programme.

3. Increases in lactate, pyruvate and plasma FFA during exercise were less after training. Increases in glycerol and glucose were greater. There was a smaller increase in the post-exercise concentration of blood ketone-bodies after training.

4. Plasma levels of insulin and growth hormone (HGH) were lower after training. The fall in insulin and the rise in HGH during exercise were also smaller.

5. The changes observed in the longitudinal study of training imply that differences observed in cross-sectional studies of athletes and untrained subjects are not the result of an innate difference but do depend upon metabolic changes related to athletic training.

CHAPTER 4

PLASMA CATECHOLAMINE CONCENTRATIONS DURING GRADED EXERCISE BY UNTRAINED SUBJECTS AND RACING CYCLISTS

4.1 INTRODUCTION

The work presented in the previous chapters provides evidence of true metabolic and hormonal differences between trained and untrained individuals, even when the trained subjects worked harder. This study was therefore designed to discover possible differences in plasma catecholamine concentration between untrained subjects and racing cyclists working at similar relative levels by the use of a more sensitive differential assay of adrenaline and noradrenaline (Appendix II). The results were to be related to changes in metabolites and hormones in blood taken during the same investigations.

Plasma concentrations of catecholamines in man have been shown to rise during exercise (von Euler, 1967; Häggendal, Hartley & Saltin, 1970). Physical training has been shown to lower the resting concentration of noradrenaline in man (Carlsson, Dencker, Grimby & Häggendal, 1968) and studies on rats showed a smaller increase in catecholamine excretion during exercise by trained animals (Östman, Sjöstrand & Swedin, 1972). Although plasma catecholamine concentration may be lower in trained than untrained subjects at a given submaximal work load it has been suggested that the plasma levels of catecholamines are similar during exercise at the same relative work load (Häggendal et al., 1970). However, Häggendal and his co-workers compared only one well-trained subject with four untrained individuals. Furthermore, their method of analysis of catecholamines did not discriminate accurately between noradrenaline and adrenaline and they were unable to measure changes in adrenaline concentration with exercise. It is, therefore, possible that true differences were obscured by practical difficulties and the use of a new highly sensitive method would provide new information.

4.2 SUBJECTS (Table 4:i)

The investigation was explained to the subjects and their consent obtained. Six racing cyclists (males aged 22-27 yr) agreed to take part in the study. They were tested at the end of a racing season during which they had trained by cycling about 200 miles per week and had frequently competed in local and national events. Details of the subjects are shown in Table 4:i. The cyclists had a mean height of 175 $\pm$ 3 cm and a mean weight of 68 kg $\pm$ 4 kg. Six control subjects (males aged 25-33 yr) were also studied. They did not take part in regular athletic training, had a mean height of 183 $\pm$ 4 cm and a mean weight of 74 $\pm$ 4 kg. The cyclists were, therefore, slightly younger, shorter and lighter than the untrained subjects.

4.3 PROCEDURE

All subjects were studied on two occasions between 1700 and 1900 hr. They had not eaten since taking a meal at mid-day. On the first occasion the work capacity of each subject was assessed by an increasing work rate test (Spiro, Juniper, Bowman & Edwards, 1973). The subject exercised on an electrically braked bicycle ergometer (Elema-Schönander) at a work load of 100 kpm/min at 60 rpm for one minute before the load was increased by 100 kmp/min each minute until they were unable to continue. On the second occasion the subjects worked for four periods of 8 min each at successive work loads estimated to be 30, 45, 60 and 75% of their maximum achieved work loads.

Heart rate was recorded on both occasions using an electrocardiograph (Elema-Schönander Mingograf).

TABLE 4:i Details of subjects, maximum work rates achieved in work rate test, resting heart rates and heart rates at maximal work rate.

(a) Cyclists

Subject	Age (yr)	Height (cm)	Weight (kg)	Maximum Work Rate (kpm/min)	Resting Heart Rate (beats/min)	Heart Rate at Maximum (beats/min)
M.D.	27	182	79	2500	58	193
D.M.	27	168	62	2100	48	195
A.T.	27	185	80	2300	96	192
W.M.	26	168	60	1900	70	196
E.McD.	22	172	67	2100	55	198
A.McD.	26	175	63	1900	48	198
Means ± SEM	26 ±0.8	175 ±3.0	69 ±4.0	2133 ± 95	63 ± 7	195 ± 1

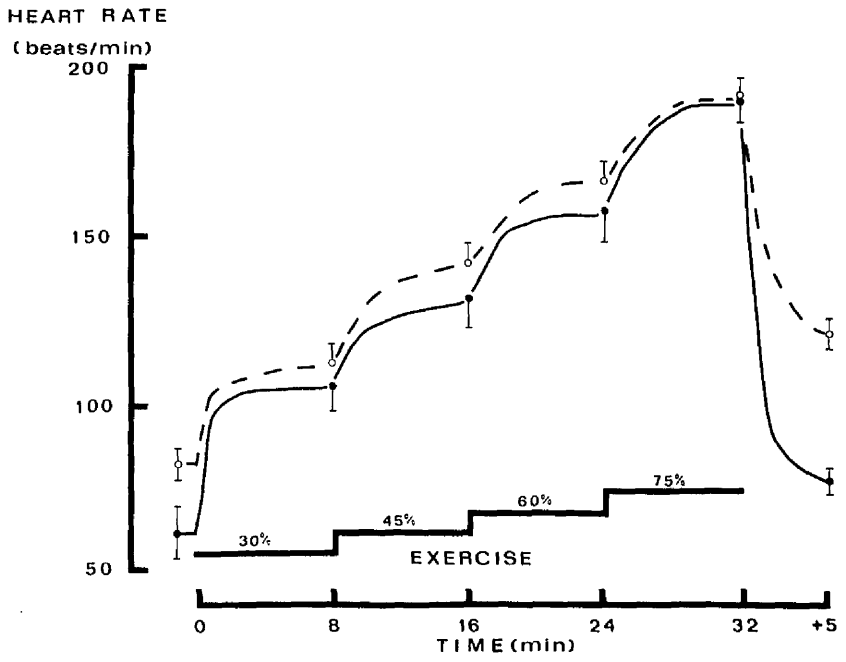
(b) Untrained Subjects

W.G.	25	185	65	1400	84	190
M.R.	27	198	90	1600	88	182
S.H.	28	186	64	1700	81	185
S.S.	28	182	78	1500	94	192
W.S.	33	168	75	1200	85	182
D.P.	33	182	70	1400	67	185
Means ± SEM	29 ±1.0	184 ±4.0	74 ±4.0	1467 ± 71	83 ± 4	186 ± 2

Blood samples were taken via an indwelling catheter previously placed in an arm vein. The catheter was flushed with physiological saline between taking samples. Samples were taken at rest sitting on the ergometer at the end of each 8 min period of exercise and 5 min after the end of the fourth period. Two samples of blood were taken on each occasion. The first (14 ml) was divided into a 4 ml aliquot which was deproteinised with 10% (w/v) perchloric acid, and a 10 ml aliquot delivered into a heparinised tube for later separation of plasma. The second sample (20 ml) for catecholamine assay was taken immediately after the first and delivered into an ice-cold Universal glass bottle containing EDTA (50 mg), sodium metabisulphite (50 mg) and pargyline (0.5 mg). The tube was capped and inverted half a dozen times. All samples were kept in ice water until the end of the investigation (37 min in all) when the deproteinised extract and blood plasma could be separated by centrifugation at 3000 rpm for 20 min. The plasma for catecholamine assay was centrifuged at 4°C. All separated samples were stored at -20°C until they could be analysed.

The deproteinised extract was used for estimation of lactate, pyruvate, acetoacetate, 3-hydroxybutyrate and glucose (see Appendix I). The separated heparinised plasma was used for estimation of cortisol, FFA, immunoreactive insulin and human growth hormone (Appendix I). The plasma from the second sample was analysed for adrenaline and noradrenaline by a two stage chromatographic purification followed by fluorimetric assay using the trihydroxyindole method (see Appendix II). All metabolites and catecholamine samples were analysed within 24 hr of sampling. Statistical analysis was performed using the

Fig. 4:1 Mean heart rate changes (beats/min) during and after graded exercise at 30, 45, 60 and 75% of maximum work level in 6 trained cyclists (● — ●) and 6 untrained subjects (o --- o). Vertical bars represent 2 SEM.


HEART RATE
(beats/min)
200
150
100
50
30%
45%
60%
75%
EXERCISE
0
8
16
24
32
+5
TIME (min)

Student's t test and the Mann-Whitney u test (Mann & Whitney, 1947).

4.4 RESULTS

4.4.1 WORK CAPACITY (Table 4:i)

The cyclists achieved a much greater maximum work output in the increasing work rate test than the untrained subjects. The difference was highly statistically significant ($P < 0.001$).

4.4.2 HEART RATE (Table 4:i; Fig. 4:i)

The cyclists had significantly lower ($P < 0.01$) heart rates at rest and significantly higher ($P < 0.01$) heart rates at the maximum achieved work load in the increasing work rate test than the untrained subjects.

When the subjects exercised at the same percentage of their maximum achieved work rate, there were no significant differences between the two groups during exercise. However, the cyclists showed a faster fall in heart rate after exercise so that they had a much lower heart rate than the untrained subjects within one minute of the end of exercise ($P < 0.001$).

4.4.3 METABOLITES IN BLOOD (Table 4:ii)

The changes in blood metabolites presented in Table 4:ii may be summarised as follows:-

(i) Resting values of all metabolites measured were similar in both groups.

(ii) Blood glucose and glycerol concentrations increased significantly more in the cyclists with exercise than in the untrained subjects.

Table 4:ii Changes with graded exercise of increasing severity (30,45,60 and 75% of maximal capacity) in blood metabolite concentrations in 6 well-trained racing cyclists and 6 untrained subjects. Values given are means ± SEM. Statistical significance was tested by the Mann-Whitney u test (NS- not significant).

Metabolites in Blood	Subjects	EXERCISE					
		0 (min)	8 (min)	16 (min)	24 (min)	32 (min)	+5 (min)
Blood Glucose (mg/100 ml)	Untrained subjects	88.0 ± 4.0	92.0 ± 5.0	89.0 ± 7.0	85.0 ± 7.0	97.0 ± 9.0	108.0 ± 10.0
	Cyclists	89.0 ± 5.0	100.0 ± 4.0	108.0 ± 5.0	116.0 ± 7.0	124.0 ± 10.0	137.0 ± 9.0
	Stat. significance	NS	NS	P<0.01	P<0.001	P<0.001	P<0.001
Blood Lactate (µmol/ml)	Untrained subjects	1.02 ± 0.14	2.21 ± 0.52	3.65 ± 0.83	5.71 ± 0.98	8.41 ± 0.97	8.83 ± 1.11
	Cyclists	0.79 ± 0.14	1.08 ± 0.23	0.98 ± 0.23	1.72 ± 0.37	5.04 ± 1.13	3.93 ± 0.91
	Stat. significance	NS	P<0.05	P<0.01	P<0.001	P<0.01	P<0.001
Blood Pyruvate (µmol/ml)	Untrained subjects	0.054 ± 0.004	0.081 ± 0.007	0.116 ± 0.013	0.153 ± 0.007	0.197 ± 0.001	0.272 ± 0.025
	Cyclists	0.051 ± 0.006	0.060 ± 0.001	0.075 ± 0.017	0.085 ± 0.025	0.147 ± 0.015	0.141 ± 0.011
	Stat. significance	NS	P<0.05	P<0.01	P<0.001	P<0.01	P<0.001
Blood Glycerol (µmol/ml)	Untrained subjects	0.096 ± 0.010	0.098 ± 0.01	0.099 ± 0.01	0.112 ± 0.013	0.119 ± 0.016	0.158 ± 0.020
	Cyclists	0.083 ± 0.003	0.120 ± 0.012	0.166 ± 0.027	0.213 ± 0.028	0.273 ± 0.037	0.308 ± 0.031
	Stat. significance	NS	P<0.05	P<0.01	P<0.001	P<0.001	P<0.001
Plasma FFA (µequiv/ml)	Untrained subjects	1.020 ± 0.087	1.035 ± 0.120	1.039 ± 0.011	1.120 ± 0.090	1.300 ± 0.090	1.690 ± 0.160
	Cyclists	0.960 ± 0.040	0.920 ± 0.060	0.990 ± 0.020	1.020 ± 0.040	1.020 ± 0.050	1.020 ± 0.070
	Stat. significance	NS	P<0.05	P<0.05	NS	P<0.01	P<0.001
Blood Ketone-Bodies (µmol/ml)	Untrained subjects	0.133 ± 0.031	0.123 ± 0.027	0.101 ± 0.021	0.086 ± 0.017	0.080 ± 0.013	0.127 ± 0.018
	Cyclists	0.171 ± 0.062	0.149 ± 0.035	0.115 ± 0.026	0.114 ± 0.023	0.100 ± 0.020	0.135 ± 0.018
	Stat. significance	NS	NS	NS	NS	NS	NS

(iii) Blood lactate, pyruvate and plasma FFA increased by smaller amounts during exercise in the cyclists than in the untrained subjects.

(iv) Although the resting concentration of ketone-bodies was higher at rest the difference was not significant. Blood ketone-bodies fell during exercise by similar amounts in both groups and rose slightly 5 min afterwards. The post-exercise rise was significantly greater in the untrained subjects than in the cyclists (Untrained 59%, Cyclists 35%; means $P < 0.05$).

4.4.4 HORMONES IN BLOOD (Table 4:iii; Fig. 4:ii)

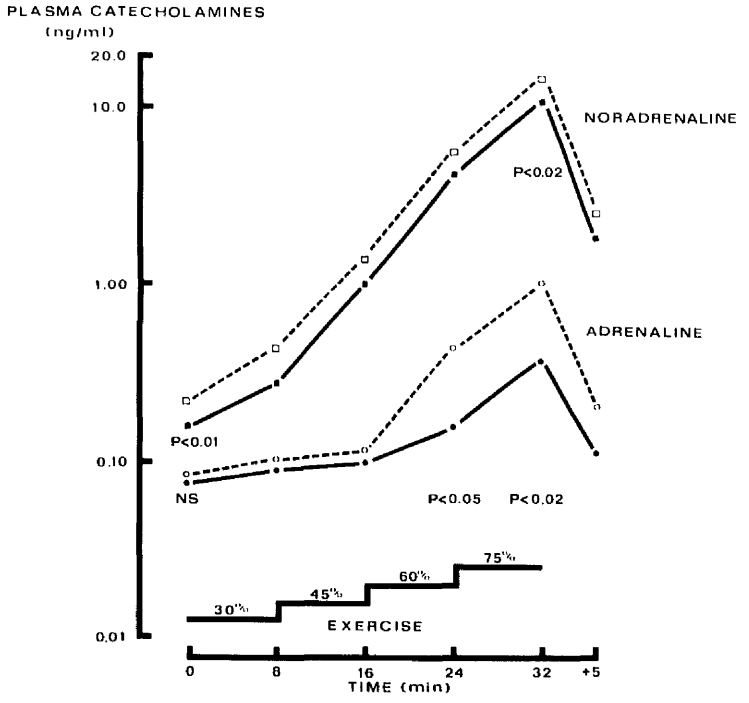
(a) Plasma IRI: Resting values of plasma insulin concentration were significantly less in the cyclists than in the untrained individuals ($P < 0.05$). During exercise insulin concentration decreased progressively with increasing work rate but the fall was significantly less in the cyclists ($P < 0.01$). Plasma insulin values rose very markedly (about five fold) 5 min after exercise in the untrained subjects but the rise was much less in the cyclists (about one half). The difference was highly significant ($P < 0.001$).

(b) Plasma HGH: Resting values of growth hormone were similar in both groups. However, during exercise the plasma HGH concentration increased more rapidly in the untrained subjects than in the cyclists. The values were significantly higher at 8 min ($P < 0.05$) and throughout the remainder of the exercise periods ($P < 0.001$). The growth hormone concentration fell after exercise in the cyclists but continued to rise in untrained subjects and the difference 5 min after the end of exercise was highly significant ($P < 0.001$).

Table 4:iii Changes with graded exercise of increasing severity (30,45,60 and 75% of maximal capacity) in plasma hormone concentrations in 6 well-trained racing cyclists and 6 untrained subjects. Values given are means ± SEM. Statistical significance was tested by the Mann-Whitney u test (NS - not significant). NB Figures for adrenaline and noradrenaline for 4 only of the cyclists.

Hormone	Subjects	EXERCISE					
		0 (min)	8 (min)	16 (min)	24 (min)	32 (min)	+5 (min)
Plasma IRI (μ units/ml)	Untrained subjects	9.0 ± 1.0	6.7 ± 0.9	5.7 ± 0.7	5.1 ± 0.8	5.0 ± 0.8	24.0 ± 3.7
	Cyclists	8.5 ± 0.5	8.4 ± 0.5	8.0 ± 0.4	7.4 ± 0.3	7.3 ± 0.3	11.0 ± 1.0
	Stat. significance	NS	P<0.05	P<0.01	P<0.01	P<0.01	P<0.001
Plasma HGH (μ units/ml)	Untrained subjects	5.4 ± 0.8	7.0 ± 1.3	17.9 ± 2.7	33.1 ± 3.5	46.0 ± 5.7	51.5 ± 6.4
	Cyclists	4.6 ± 0.5	5.2 ± 0.6	8.3 ± 1.3	14.6 ± 1.8	22.6 ± 1.9	18.2 ± 1.9
	Stat. significance	NS	P<0.05	P<0.001	P<0.001	P<0.001	P<0.001
Plasma Cortisol (μg/ml)	Untrained subjects	10.7 ± 0.6	6.6 ± 0.8	4.8 ± 0.4	7.1 ± 0.5	17.5 ± 0.8	17.0 ± 1.3
	Cyclists	9.6 ± 0.7	8.5 ± 0.7	7.0 ± 0.4	14.2 ± 1.8	26.2 ± 3.2	19.5 ± 2.0
	Stat. significance	NS	NS	P<0.01	P<0.001	P<0.001	NS
Plasma Noradrenaline (ng/ml)	Untrained subjects	0.215 ± 0.015	0.423 ± 0.047	1.36 ± 0.17	5.50 ± 0.61	13.70 ± 1.42	2.61 ± 0.30
	Cyclists	0.157 ± 0.012	0.270 ± 0.020	0.98 ± 0.12	4.05 ± 0.32	10.66 ± 0.90	1.83 ± 0.41
	Stat. significance	P<0.05	P<0.05	P<0.05	P<0.02	P<0.01	P<0.02
Plasma Adrenaline (ng/ml)	Untrained subjects	0.08 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.44 ± 0.10	1.02 ± 0.15	0.19 ± 0.03
	Cyclists	0.08 ± 0.01	0.08 ± 0.02	0.09 ± 0.01	0.16 ± 0.09	0.37 ± 0.08	0.10 ± 0.02
	Stat. significance	NS	NS	NS	P<0.05	P<0.01	P<0.02

Fig. 4:ii Mean plasma catecholamines (adrenaline and noradrenaline; ng/ml) during and after graded exercise at 30, 45, 60 and 75% of maximum work level in 4 trained cyclists (● —— ●) and 6 untrained subjects (o ·---·o). Statistical significance assessed by Mann-Whitney u test.


PLASMA CATECHOLAMINES
(ng/ml)
20.0
10.0
1.00
0.10
0.01
NORADRENALINE
ADRENALINE
P<0.02
P<0.01
NS
P<0.05
P<0.02
30'%
45'%
60'%
75'%
EXERCISE
0
8
16
24
32
+5
TIME (min)

(c) Plasma cortisol: Cortisol concentration at rest was similar in both groups. During exercise cortisol levels were decreased at 8 min and 16 min but rose above resting in all subjects at 24 and 32 min. The rise was significantly greater ($P < 0.01$) in the racing cyclists. In all subjects the plasma cortisol concentrations were decreased 5 min after exercise and the values of the cyclists were only marginally elevated above those of the untrained subjects.

(d) Plasma catecholamines: Resting concentrations of noradrenaline were lower ($P < 0.05$) in the racing cyclists but there was no significant difference between the two groups in adrenaline concentrations.

In both groups noradrenaline concentration increased exponentially as the subjects exercised against the increased loads. The rise was smaller in the cyclists, however, and they had significantly lower plasma noradrenaline concentrations at the end of each work period ($P < 0.01$).

The untrained subjects also showed a greater rise in adrenaline at the two highest work loads. The cyclists had only small increases in adrenaline concentration during exercise except at the highest work load when the value was significantly lower than in the untrained subjects.

In both groups catecholamine concentrations fell rapidly after exercise to values at 5 min afterwards of about one third of the maximum values at the end of exercise. The adrenaline and noradrenaline concentrations remained significantly higher in the untrained group compared to the cyclists ($P < 0.05$).

4.4 DISCUSSION

There was a large overlap between the two groups of subjects although they were not matched exactly for age, height and weight. The cyclists were slightly younger, shorter and lighter but it is unlikely that the small differences between the groups were reflected in the results. Although any such effects would be expected to exaggerate the differences between the well-trained and untrained subjects no relation could be found between age, height, weight and, for example, blood lactate concentration for individuals within each group.

The heart rate changes demonstrate the well known cardiovascular adaptations to exercise which occur in trained athletes (Åstrand et al.,1968; Edwards et al., 1969). The cyclists had much lower heart rates at rest, they increased their heart rates more quickly at the beginning of exercise and at each new work level and they showed a much faster recovery after exercise.

The test used for assessing working capacity in all the subjects was empirical (Spiro et al., 1973) and although heart rate was measured, oxygen consumption was not. However, the operational validity of the test was borne out by the similar heart rates achieved by both groups of subjects at each level of exercise, suggesting that there were no differences in the relative work loads for the two groups (Spiro et al.,1973; Åstrand et al., 1968).

Resting values of blood glucose, lactate and pyruvate were similar in the two groups and within the range normally encountered. However, in both groups the FFA and blood ketone-body concentrations

were higher at rest than observed in previous investigations. All previous investigations were carried out in the morning with overnight fasted subjects but the present study was carried out between 1700 and 1900 hr with subjects who had completed a day's work and had eaten last at mid-day. Blood glycerol values were normal excluding increased lipolysis as an explanation. It is possible that the higher concentrations of FFA and ketone-bodies might be due to fat taken as food and its subsequent low oxidation in liver following absorption.

The changes in metabolites in the blood during and immediately after exercise in the present study confirm the conclusions reached in Chapters 2 and 3 and discussed there. True metabolic differences exist in the response to exercise in well-trained and untrained individuals at the same relative work load. These differences are shown in both the mobilisation and utilisation of fuels for muscular work. Thus the present results show a much greater increase with exercise in blood glucose concentration in the racing cyclists but they, nevertheless, have lower blood concentrations of lactate and pyruvate. The differences between the well-trained and the untrained subjects are more marked for fat metabolites. There was an immediate sustained mobilisation of stored fat with exercise in the cyclists in whom blood glycerol concentration tripled during the exercise period. Nevertheless, plasma FFA concentration hardly increased. The untrained subjects showed a limited increase in blood glycerol concentration (about 20% at the end of exercise) during exercise but values of plasma FFA increased by almost 70%. There was also a significantly greater increase in ketone-body concentration 5 min after

e xercise in the untrained group. These results suggest that the biochemical adaptation towards efficient oxidative muscle metabolism following athletic training is greater for fat than for carbohydrate. A discussion of the biochemical mechanisms appears in Chapters 2 and 3.

Previous work presented in this thesis suggest that the hormonal response to exercise is different in well-trained a nd untrained individuals (Chapters 2 and 3). Insulin has been shown to be greater in untrained subjects at rest and during exercise than in well-trained subjects (Bjorntorp et al., 1970 & 1972) and growth hormone to be higher during exercise in unfit individuals (Sutton, et al., 1968; 1969) The results of the present study confirm these findings. In addition the results show that there is a fall in plasma insulin and a rise in p lasma growth hormone during exercise which are greater in the untrained subjects than in the racing cyclists. The magnitude of the changes in each group appears to be related to the intensity of the exercise. The present results show for the first time that these differences remain even when the athletically trained subjects and the unfit subjects are working at similar relative levels; that is when the trained individuals work much harder in absolute terms.

The mechanisms of these changes during exercise are not understood. The fall of insulin is not related to changes in b lood glucose, which rises in both groups. The greater rise in blood glucose in the cyclists might have prevented a large fall in insulin but this is unlikely since the racing cyclists also showed a much smaller rise in insulin after exercise when they had h igher blood glucose values. The extraction of insulin by

working muscle depends on its metabolic requirements (Wahlqvist et al., 1972a) and since the well-trained and untrained subjects show apparently different patterns of fat and carbohydrate metabolism in exercise this might partially explain the present differences in plasma insulin concentration. The changes can not be explained by a rise in FFA during exercise since insulin secretion is stimulated by FFA and ketone-bodies. However, the very large rise in insulin 5 min after exercise in the untrained subjects might be explained by the large post-exercise increase in plasma FFA.

The rise in HGH during exercise is also not well understood. (Sutton et al., 1969). Since there was no fall in blood glucose in either group, hypoglycaemia can not be implicated as suggested by Hunter and co-workers (Hunter, Fonseka & Passmore, 1965). Sutton et al (1969) have suggested that lactate may stimulate HGH secretion and reports increases after infusion of lactate. The greater blood lactate concentration in unfit subjects might explain their greater increase of HGH during exercise. However, no change in HGH levels could be shown with lactate infusion in six subjects investigated recently (Rennie, unpublished results).

Changes in plasma cortisol have previously been demonstrated during and after exercise. Moderate exercise (about 600 kpm) has been shown to decrease cortisol levels in man (Cornil, De Coster, Copinschi & Franckson, 1965). Sutton and co-workers (1969) showed an initial fall of plasma cortisol during exercise followed by a rise and the present results show a similar pattern. The initial fall might be the result of uptake of cortisol by skeletal muscle (Cornil et al., 1965; Wahlqvist et al., 1972b).

Sutton was unable to demonstrate a significant difference in cortisol levels between fit and unfit subjects. The present results show significantly higher values of plasma cortisol during exercise in well-trained individuals than in untrained persons working at the same relative load. It is unknown whether or not the raised cortisol observed during the work loads of high intensity was due to an increase of secretion or to a decrease of peripheral uptake, although the second possibility seems unlikely. Frankl and Csalay (1962) found an increased capacity for cortisol secretion in well-trained rats so that greater secretion of cortisol by the racing cyclists is the most probable explanation of the differences between the two groups at high levels of exercise.

The metabolic effects of cortisol and its effects on other hormones during exercise are difficult to assess. Cortisol antagonises the insulin induced transport of glucose into muscle and increases liver gluconeogenesis (Newsholme & Randle, 1964). It does not seem, however to have a direct effect on insulin secretion. The rise observed in glucose with exercise cannot be related to decreased uptake of glucose by muscle due to increases in cortisol concentration since blood glucose rises as cortisol is initially falling. Although cortisol has a long-term lipolytic effect at rest the metabolic effects of cortisol on fat metabolism during severe exercise are unknown. However, no difference during moderate exercise could be observed in fat metabolism between hypopituitary patients receiving cortisol replacement and those who were not (Johnson, Rennie, Walton & Webster, 1971; see Chapter 7). Nevertheless, the higher plasma cortisol concentrations observed in the well-trained

subjects during exercise may have contributed to increased lipolysis observed as a greater release of glycogen since cortisol inhibits FFA re-esterification (Shafir & Kerpel, 1969).

The changes in plasma cortisol cannot explain the differences in HGH during exercise since although both cortisol and HGH may rise as a result of stress (Zahnd, Nadeau & van Mühlendahl, 1969), the greater rise of HGH was observed in the untrained subjects, who showed a smaller increase in cortisol. Also Greenwood and London (1966) described a rise in cortisol in male subjects after infusion of lysine-vasopressin without a concomittant rise in HGH.

Many of the mechanisms suggested for mobilisation of fuel and alteration of hormone secretion during exercise involve catecholamine mediated activity. This may be either directly, through the sympathetic innervation of tissues and the release of noradrenaline from nerve endings or via stimulation of the adrenal medulla to release adrenaline (Blackard & Hubbell, 1970; Cronin, 1967; Gollnick, Soule, Taylor, Williams & Ianuzzo, 1970; Riddle, Ryan & Schwartz, 1972; Sutton et al., 1968; Young, Pelligra & Adachi, 1966; von Euler, 1969). However, because of severe methodological difficulties in the measurement of both adrenaline and noradrenaline in blood, most of the published information has depended on studies using alpha and beta blocking agents and adrenalectomy in animals. The present study was designed to delineate the changes in both adrenaline and noradrenaline in man during exercise and to discover the influence on them

of physical fitness. A further aim was to study the role of adrenergic mechanisms in the regulation of metabolism and in secretion of other hormones during exercise.

The results show significantly lower plasma concentrations of noradrenaline at rest in the well-trained subjects. No significant difference could be seen in adrenaline concentrations, probably because of the great difficulties of measuring the small amounts of amines. Both noradrenaline and adrenaline show an increase during exercise but the rise in adrenaline is detectable only at high work levels. Similar results have been reported by other workers (Häggendal et al.,1970; Kotchen, Hartley, Rice, Mougey, Jones & Mason, 1971). The present results also demonstrate significantly lower concentrations of noradrenaline and adrenaline in well-trained individuals working at the same relative work levels as untrained persons. This observation has not previously been described by other workers. However, the present study was specifically designed to detect possible differences between subjects of different physical fitness and the method used for catecholamine assay is more sensitive, particularly for adrenaline, than those used by other workers (see Appendix II).

The significance of increased concentrations of circulating catecholamines during exercise is not clear. Adrenaline concentration in plasma probably reflects secretion from the adrenal medulla and there will be a direct relationship between the plasma value and metabolic and physiological effects. However, the concentration of circulating noradrenaline reflects the amount of release from sympathetic nerve terminals and a distinction must be drawn between the metabolic and physiological effects of sympathetic nervous activity and the effects of

noradrenaline in the blood. Also circulating levels must reflect a net flux of catecholamines released from nerve endings and the adrenal gland and also taken up again by nerve terminals and liver and other tissues (Iversen, 1967). The plasma concentrations might thus be decreased considerably by increased liver and muscle blood flow during exercise by athletically trained individuals (Ekblom, Åstrand, Saltin, Stenberg & Wallström, 1968; Rowell, 1969).

Results obtained from infusion studies in man with plateau levels of 1.5 µg catecholamines/litre (Häggendal, 1971) and from animal studies (Folkow, Löfving & Mellander, 1956) suggest that control of cardiovascular functions occurs predominantly via the noradrenergic nerves, which directly innervate heart and blood vessels. The role of blood borne noradrenaline appears to be minor. The present results support this since although the heart rate in both groups of subjects fell rapidly after exercise the half-time of plasma catecholamine concentrations was significantly longer ($P < 0.05$). The lower resting heart rate in the racing cyclists is more likely to be related to hypertrophic changes of the heart than to the lower values of circulating noradrenaline.

With respect to the metabolic effects of catecholamines the situation is less clear. Studies on electrical stimulation of sympathetic nerves to canine subcutaneous adipose tissue show that nervous activity is more effective in the mobilisation of fat than circulating catecholamines (Rosell & Ballard, 1971). Also individual skeletal muscle cells appear to lack direct sympathetic innervation although blood vessels of skeletal muscles contain adrenergic nerves (Fuxe & Sedvall, 1965). Nevertheless,

noradrenaline may diffuse across the short distances from nerve terminals and be present in locally high concentration in muscle cells. The concentrations of circulating adrenaline measured at rest and during exercise in the present study are high enough to affect glycogenolysis in liver and muscle and lipolysis of adipose tissue (Exton, Mallette, Jefferson, Wong, Friedmann, Miller & Park, 1970). Although noradrenaline is metabolically less active than adrenaline (Goldenberg, Aranow, Smith & Faber, 1950), the circulating concentrations measured in the present study during exercise are probably high enough to exert some effect. However, evidence is accumulating from a number of sources that catecholamines do not necessarily have a direct effect on fuel mobilisation. The exercise induced rise in plasma FFA is not suppressed by beta-blockade in rats (Gollnick, 1967; Gollnick et al., 1970; Lefebvre, Luyckx & Federspil, 1972) and in man (Cärlsten, Häggendal, Hallgren, Jagenburg, Svanborg & Werko, 1968). Also propranolol does not block glycogenolysis in muscle during exercise (Harris, Bergström & Hultman, 1971). The mechanism of a direct effect of circulating catecholamines on fuel mobilisation would be difficult to understand in the light of the present findings. Noradrenaline and adrenaline concentration during exercise are lower in the well-trained cyclists in whom the mobilisation of liver glycogen and body fat was apparently greater than in the untrained subjects.

Lefebvre and co-workers (1972) have postulated that the lipolytic effect of endogenous catecholamines on fat mobilisation is mediated through inhibition of insulin release. The exercise induced inhibition of insulin release, resulting in a fall in

peripheral plasma concentration is abolished by alpha-blocking agents such as phentolamine (Brisson, Malaisse-Lagae & Malaisse, 1971) but is not suppressed by beta-blockers (Lefebvre et al., 1972). This is supported to some extent by the present results which show a greater fall in plasma IRI in the untrained subjects, who have a larger increase in catecholamines during exercise, particularly of the more metabolically active adrenaline, which has both alpha and beta-stimulatory activity. However, this interpretation is again complicated by the present observation that the untrained subjects showed a greater suppression of insulin but a lower degree of lipolysis judged by the index of glycerol release (but see discussion of effect of lactate on lipolysis in Chapters 2, 5 and 6).

The hormonal and metabolic interactions are obviously very complex and more information is needed. No data exists, for example, on the effect of insulin on sympathetic activity although high levels of total catecholamines in ketotic juvenile diabetics have been shown to fall after insulin treatment (Christensen, 1970). Similarly, there is little information available on interaction during exercise of insulin, catecholamines and glucagon, which stimulates lipase activity in adipose tissue. (Birnbaumer & Rodbell, 1969).

Many of the present findings such as apparently increased lipolysis and lower levels of catecholamines might be explained if the well-trained subjects were more sensitive to the metabolic effects of catecholamines, either released at nerve terminals or circulating in the blood. There is some evidence that cortisol increases sensitivity of tissues to catecholamines (Gollnick et al.,

1970) and the greater levels of cortisol at higher work loads might have had this effect in the racing cyclists. The present findings of lower catecholamine levels in athletically trained subjects who also show a smaller rise in HGH might provide evidence for the suggestion of Blackard and Hubbell (1970) that release of HGH by circulatory catecholamines is increased (see also Troyer, Friedberg, Horton & Bogdonoff, 1966).

The post-exercise changes in catecholamines are similar to those reported elsewhere (Kotchen et al., 1971; Vendsalu, 1960). The differences seen in both adrenaline and noradrenaline in the cyclists and the untrained controls are preserved after exercise and the continuing higher levels of catecholamines in the untrained subjects at rest might contribute to their sudden increase of lipolysis after exercise (see also Chapters 1, 2 and 3) and hence to the development of post-exercise ketosis. However, a more likely explanation of this is the lower oxidation capacity for fat metabolism and possibly for re-esterification in the untrained subjects. If a low level of re-esterification does occur after exercise in the untrained subjects then the effect must be relatively insensitive to insulin, which rises considerably after exercise in the unfit subjects. Another factor which would cause lipolysis after exercise in the unfit subjects is the continued rise of HGH and cortisol which have a synergistic effect on fat metabolism and antagonise the actions of insulin on adipose tissue. Both hormones fall after exercise in the athletically fit subjects, who do not normally develop post-exercise ketosis (see Chapter 2).

The present results demonstrate that there are differences in the regulation of metabolic and hormonal changes during exercise in

athletically trained and untrained individuals even when the trained subjects work at the same relative level. In particular these observations demonstrate for the first time that trained athletes secrete lower levels of catecholamines at any relative level of exercise, either submaximal or maximal, than untrained subjects.

4.5 SUMMARY

1. Six well-trained cyclists and six untrained subjects were studied during and immediately after four successive 8 min periods of exercise at 30, 45, 60 and 75% of their maximal work capacity.

2. Venous blood samples were taken at rest, at the end of each exercise level and 5 min following the end of exercise, for estimation of metabolites in blood and plasma IRI, HGH, cortisol and catecholamines.

3. The results showed significant differences in the mobilisation and utilisation of muscle fuels between the athletically fit cyclists and the untrained group. In the cyclists, glucose and glycerol concentrations were higher but FFA, lactate and pyruvate were lower than in the untrained subjects during exercise.

4. There were also marked differences in the hormonal response to exercise in the two groups:

a. Plasma IRI was depressed to a greater extent in the untrained subjects during exercise and they also showed a large increase in IRI 5 min after the end of exercise.

b. Plasma HGH rose to a greater extent during exercise and remained elevated after the end of exercise in the untrained group. The racing cyclists had a smaller increase in HGH, which

fell after the end of exercise.

c. Plasma cortisol fell initially during exercise in both groups but to a smaller extent in the cyclists. Cortisol levels rose in the last two exercise periods and were significantly higher in the cyclists at the end of exercise. Plasma cortisol fell after exercise in the cyclists but remained elevated in the untrained subjects.

d. Plasma catecholamines rose in both groups during exercise but the rise was significantly less in the racing cyclists. Plasma adrenaline concentrations were, in particular, much more elevated at the end of exercise in the untrained subjects.

5. There are thus significant differences in the metabolic and hormonal response to exercise between athletically trained and untrained individuals, even when the physically fit subjects work at the same percentage of their maximal capacity as the unfit subjects. Furthermore, these results demonstrate for the first time lower circulating concentrations of noradrenaline and adrenaline in athletically trained subjects working harder in absolute terms but at the same relative level as untrained subjects.

CHAPTER 5

THE EFFECT OF DIET UPON THE METABOLIC CHANGES WITH EXERCISE IN LONG-DISTANCE RUNNERS

5.1 INTRODUCTION

By consumption of a carbohydrate-rich diet following strenuous exercise to deplete muscle stores, the glycogen concentration in working muscles can be considerably increased. The glycogen content of leg muscle falls rapidly with severe exercise and the duration of high intensity work is correlated with initial glycogen content (Hultman, 1967). Thus after a glycogen enhancing regime there is an increase in exercise tolerance so that the duration of exercise at a fixed load can be doubled (Bergström, Hermansen, Hultman & Saltin, 1967).

Although there is evidence of greater ability of working muscle to use carbohydrate as fuel, little information exists on the effects of the regime on fatty acid metabolism by working muscle. The results presented in Chapters 2, 3 and 4 suggest that well trained individuals are apparently able to use fat more efficiently as a fuel for muscular work than untrained subjects. They should, therefore, show greater changes than untrained subjects in fat supply and utilisation after a glycogen enhancing regime. A group of long-distance runners who were interested in possible benefits of the regime to their performance agreed to take part in a study of its metabolic effects during and after exercise.

5.2 PROCEDURE (Fig. 5:i)

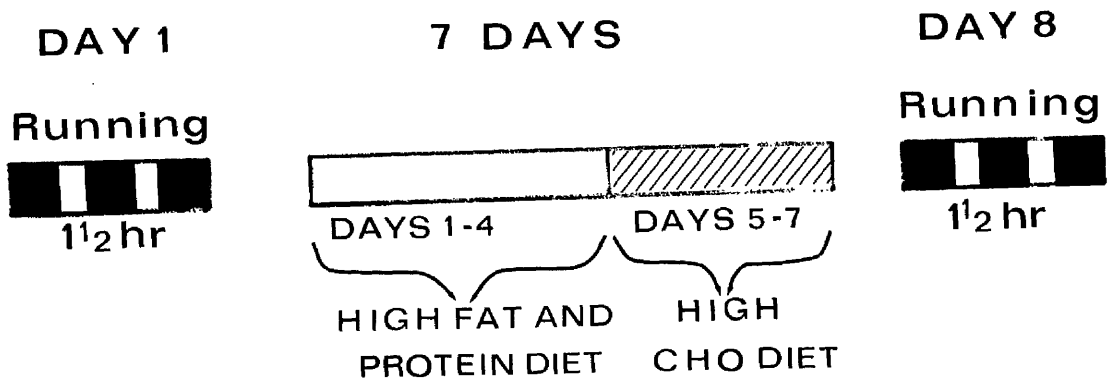
Six long-distance runners were studied (see Table 5:i for details). The investigation was explained to them and the types of diet described. They were studied on successive weekends when they ran a distance of about 15 miles at the same rate on each occasion, taking 90 min. The weather conditions

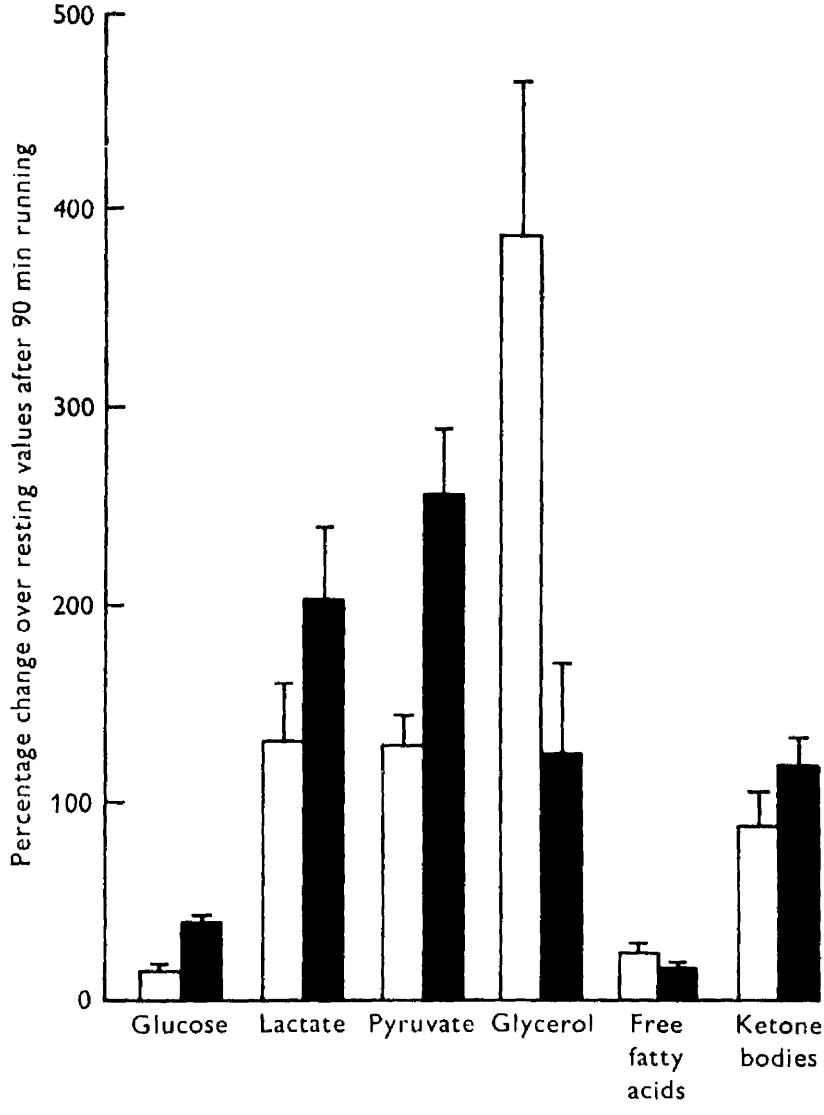
TABLE 5:i DETAILS OF LONG-DISTANCE RUNNERS

Subject	Age (yr)	Height (cm)	Weight		Comment
			Before Diet (kg)	After Diet (kg)	
N.D.	27	169	56.4	54.8	Regular training: club runner.
I.S.	15	164	52.9	55.0	West of Scotland Schoolboy Champion.
J.McL.	43	178	66.2	68.5	Good average runner.
G.P.	58	174	60.0	61.4	Runs marathon in less than 3 hr. N.B. age.
W.S.	41	172	62.0	64.0	Current World veteran 15 km champion.
D.W.	27	193	71.1	73.5	2nd in Scottish marathon in 1972.
Mean	35.2	175.0	61.4	62.8	
SEM	±6.2	± 4.1	±2.7	±3.0	

Fig. 5:i Scheme of Investigation.

Fig. 5:ii Percentage change in metabolites in blood after 90 min running in 6 long-distance runners before (open bars) and after (closed bars) a muscle glycogen enhancing regime (means $\pm$ SEM).


DAY 1
Running
1½ hr
7 DAYS
DAYS 1-4
DAYS 5-7
HIGH FAT AND PROTEIN DIET
HIGH CHO DIET
DAY 8
Running
1½ hr


Percentage change over resting values after 90 min running
500
400
300
200
100
0
Glucose
Lactate
Pyruvate
Glycerol
Free fatty acids
Ketone bodies

were similar on both days (dry, no wind, temperature 9°C, 9°C, humidity 84% and 90%). They ate a normal mixed diet before the first run, took a high fat and protein diet for 3 days and then a carbohydrate-rich diet for 3-4 days before running again. Each subject kept a diary of food intake during the week and calorific intake was calculated from the reported figures (see Tables 5:ii and 5:iii).

On each occasion venous blood was taken at rest, at 30, 60 and 90 min running and at 15-30 min intervals for 105 min afterwards. Blood was analysed for glucose, lactate, pyruvate, acetoacetate and 3-hydroxybutyrate, and glycerol. Plasma was analysed for free fatty acids (FFA) insulin and growth hormone. All analyses were carried out as described in Appendix I.

5.3 RESULTS

5.3.1 DIET (Tables 5:ii and 5:iii)

All of the runners managed to keep broadly to the diet as outlined to them and there was a remarkable similarity in the mean daily calorific intake during the high fat/high protein part of the diet and the high carbohydrate part.

5.3.2 METABOLITES IN BLOOD (Fig. 5:ii)

(a) Blood glucose: (Fig. 5:ii and 5:iii) The resting levels of blood glucose were significantly lower after the diet. (Before 99 ± 6 (SEM), after 81±7 (SEM) mg/100 ml blood) Exercise caused a rise in the blood concentration of glucose both before and after the diet but the percentage rise was greater and was

TABLE 5:ii

DETAILS OF CALORIFIC INTAKE DURING DIET

	DAYS 1 - 3 HIGH FAT/HIGH PROTEIN				DAYS 4 - 6 HIGH CARBOHYDRATE			
Subject	Average protein intake calories	Average fat intake calories	Average carbohydrate intake calories	Mean total calories	Average protein intake calories	Average fat intake calories	Average carbohydrate intake calories	Mean total calories
N.D.	921	3425	890	5236	640	2412	2317	5369
I.S.	872	3079	743	4694	583	2210	2009	4802
J.McL.	987	2877	1002	4866	675	2408	2034	5117
G.P.	843	2373	722	3938	631	1873	1844	4348
W.S.	1006	2830	877	4713	895	2143	2210	5248
D.W.	928	2758	1012	4698	775	1987	2111	4873
			Mean calories per day	4690 ±172			Mean Calories per day	4959 ±150

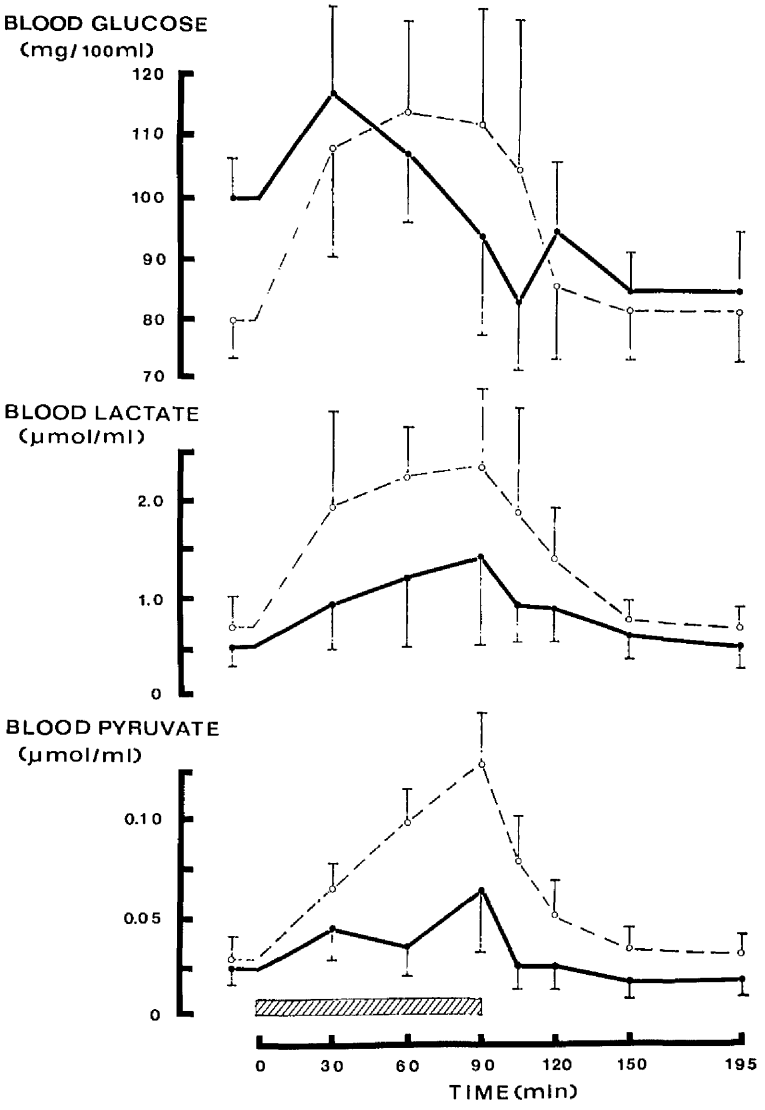
TABLE 5.iii

FOOD INTAKE OF SUBJECT J.McL. ON TWO DAYS

DAY 3 HIGH FAT/PROTEIN				
Raisins	5oz			
Ryvita	3oz			
Cheese	4oz			
Egg	2oz			
Bacon	2oz	TOTAL	Protein	975
Apple	4oz		Fat	2730
Milk	30oz		Carbohydrate	1055 cals
Kippers	12oz		i.e.	4760 cals
Soya	1oz			
Wheatgerm	1oz			
Soup	10oz			
Butter	4oz			
Steak	8oz			

DAY 6 HIGH CARBOHYDRATE				
Breakfast cereal	1oz			
Bacon	3oz			
Eggs	4oz			
Bread	14oz			
Jam	2oz	TOTAL	Protein	632 cals
Sugar	2oz		Fat	2610 cals
Steak	8oz		Carbohydrate	2032 cals
Onion	2oz		i.e.	5274 cals
Potatoes	6oz			
Carrot	1oz			
Cheese	4oz			
Biscuits	3oz			
Cake	3oz			
Milk	20oz			
Butter	4oz			

Fig. 5:iii Changes during and after 90 min running in blood glucose (mg/100 ml), blood lactate (μmol/ml) and blood pyruvate (μmol/ml) in 6 long-distance runners before (● —— ●) and after (o ---- o) a muscle glycogen enhancing regime (means ± SEM).


BLOOD GLUCOSE
(mg/100ml)
120
110
100
90
80
70
BLOOD LACTATE
(µmol/ml)
2.0
1.0
0
BLOOD PYRUVATE
(µmol/ml)
0.10
0.05
0
0
30
60
90
120
150
195
TIME (min)

sustained longer after the glycogen-enhancing regime. (Fig. 5:ii) The differences were most marked at 90 min of running and at 15 min after running had ceased when the post-diet glucoses were highly significantly elevated ($P < 0.001$).

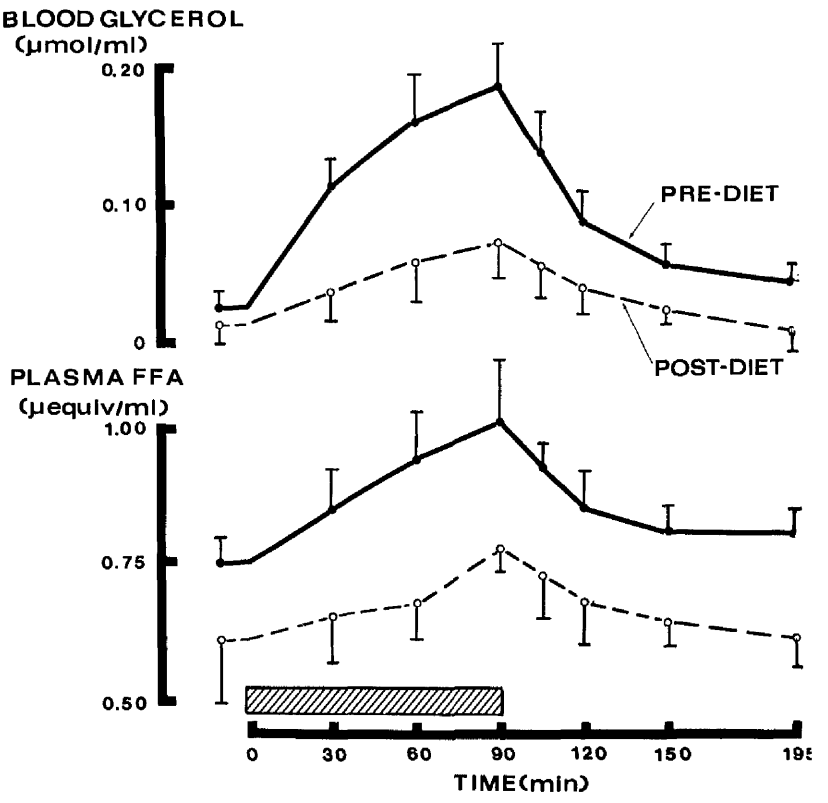
(b) Blood pyruvate and lactate: (Fig. 5:ii and 5:iii) Lactate and pyruvate concentrations were similar at rest on both occasions. However, there was a greater rise in the blood concentration of these metabolites during the post-diet run so that they were significantly greater on the basis of paired Student's t test at 30 min ($P < 0.05$), 60 min ($P < 0.01$) and 90 min ($P < 0.001$). The lactate-pyruvate ratio was also significantly greater during the second period of running at 30, 60 and 90 min.

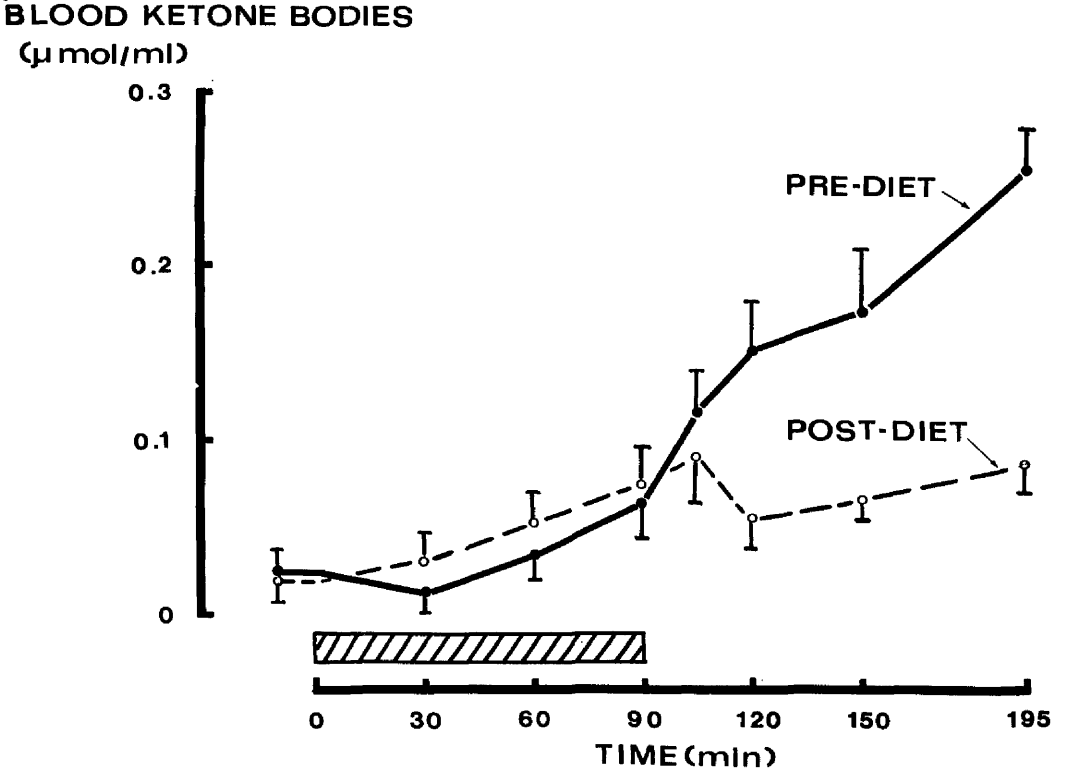
(c) Blood glycerol: (Fig. 5:ii and 5:iv) Glycerol concentrations were similar at rest on both occasions but the exercise induced rise in the blood level was very much less after the glycogen enhancing regime. The difference was most marked ($P < 0.001$) at the end of the running when the changes were 385 and 125% of the resting levels during the pre- and post-diet investigations. The rate of return to pre-exercise values was greater after the first run but the glycerol levels remained elevated compared to the concentrations after the second run.

(d) Plasma FFA: (Fig. 5:ii and 5:iv) Plasma FFA were significantly lower throughout the post-diet investigation ($P < 0.05$). The exercise induced rise in plasma FFA values was slightly greater during the first run (33%) than after the carbohydrate-rich diet (25%) and the difference was significant

Fig. 5:iv Changes during and after 90 min running in blood glycerol (μmol/ml) and plasma FFA (μequiv/ml) in 6 long-distance runners before (● — ●) and after (○ --- ○) a muscle glycogen enhancing regime (means ± SEM).

Fig. 5:v Changes during and after 90 min running in blood ketone-bodies (acetoacetate and 3-hydroxybutyrate; μmol/ml) in 6 long-distance runners before (● — ●) and after (○ --- ○) a muscle glycogen enhancing regime (means ± SEM).


BLOOD GLYCEROL
(μmol/ml)
0.20
0.10
0
PRE-DIET
POST-DIET
PLASMA FFA
(μequiv/ml)
1.00
0.75
0.50
0
30
60
90
120
150
TIME(min)


BLOOD KETONE BODIES
(μ mol/ml)
0.3
0.2
0.1
0
PRE-DIET
POST-DIET
0
30
60
90
120
150
195
TIME (min)

on the basis of a paired t test ($P < 0.05$).

The FFA-glycerol ratio was greater in the post-diet situation at 30, 60 and 90 min of running.

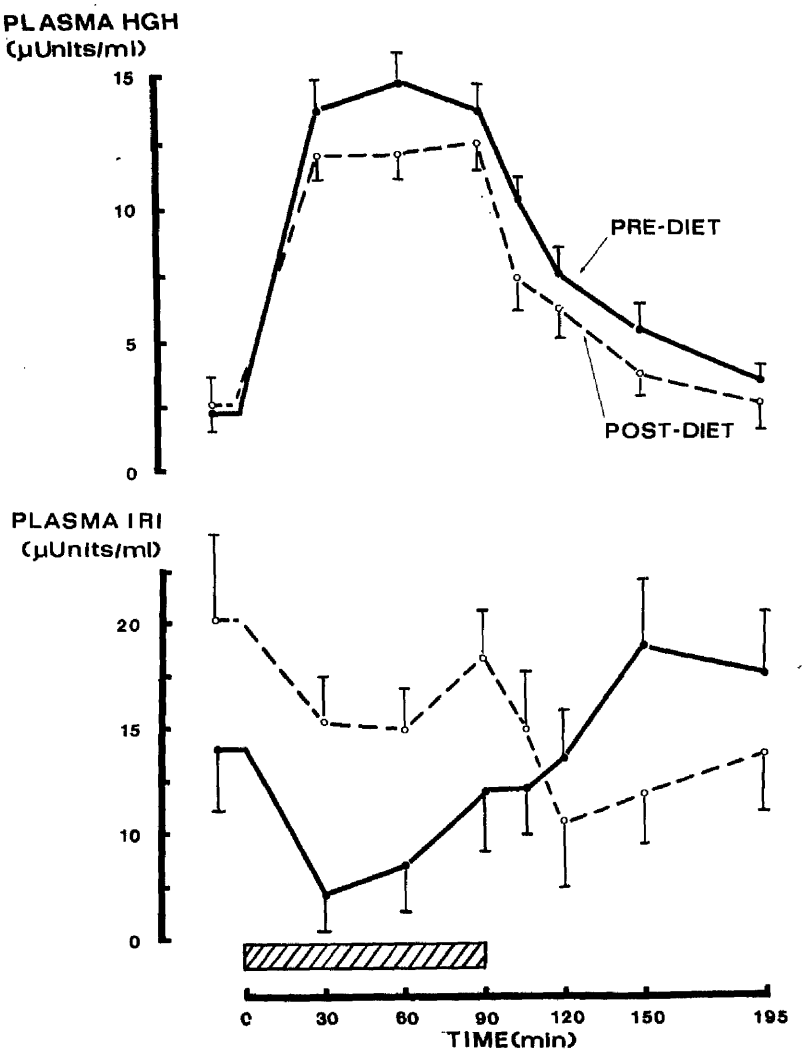
(e) Blood ketone-bodies: (Figs. 5:ii and 5:v) There was no significant difference in the pre-exercise levels of ketone-bodies on either of the two occasions but the pattern of change during running was different in the pre- and post-diet situations. Ketone-body levels fell slightly during the first 30 min of exercise on the first run and rose during the remaining hour of running and the succeeding 105 min post-exercise. The ketone-body concentrations rose without a fall during the second post-diet run and were in all subjects slightly higher during the period of running. The greater elevation of blood ketone-bodies during the period of running in the post-diet situation just attained statistical significance ($P < 0.05$, paired t test). After exercise on the second occasion ketone-body concentrations did not rise above the value attained at the end of exercise so that there were great differences between the blood levels at 105 min after exercise on the two occasions ($P < 0.001$).

5.3.3 HORMONES IN BLOOD

(a) Plasma HGH: (Fig. 5:vi) Resting levels of plasma human growth hormone were not significantly different on the two occasions but the concentrations during the period of running were higher before the diet, although only significantly so at 60 min ($P < 0.05$).

(b) Plasma Insulin: (Fig. 5:vi) Insulin levels were significantly ($P < 0.001$) higher at rest in every subject after the high carbohydrate diet. On both occasions insulin fell

Fig. 5:vi Changes during and after 90 min running in plasma HCH and IRI (μunits/ml) in 6 long-distance runners before (o —— o) and after (o ---- o) a muscle glycogen enhancing regime (means ± SEM).


PLASMA HGH
(μUnits/ml)
15
10
5
0
PRE-DIET
POST-DIET
PLASMA IRI
(μUnits/ml)
20
15
10
0
0
30
60
90
120
150
195
TIME(min)

during the period of running but the fall was sustained after exercise following the high carbohydrate diet. The insulin levels in the first run, after the normal diet, rose above the resting levels in the period of the investigation after exercise so that the concentrations were significantly different at 60 min and 105 min post-exercise.

5.4 DISCUSSION

All of the runners had some difficulty in keeping to the diet as outlined to them at the start of the investigation. In particular they tended to eat a greater amount of food during the second part of the diet when they concentrated on carbohydrate at the expense of fat and protein. This would explain the slight weight gain in the group before the second investigation. Although the diet has been reported to have a beneficial effect on the performance of athletes the effects reported by the runners in the present investigation were variable. Some of the subjects suffered from constipation during the high fat-high protein part of the diet but this cleared up before the second run. They reported feeling very fit and full of energy at the start of the second run but they felt little subjective difference in the performance of the exercise. However, this was not surprising since most of the subjects were running within their limits for, as marathon runners, they were used to running longer distances at a higher speed. Two of the subjects have subsequently tried the glycogen-enhancing regime before running competetively and were successful in improving their performance compared to previous similar outings.

It was unfortunately impossible to measure glycogen content of muscle before and after the diet since the techniques for needle biopsy were not available. Therefore the following discussion of metabolic changes as a result of the diet assume that the reported increase in muscle glycogen content also occurred in the present study. (Bergström et al., 1967)

The lower resting levels of blood glucose in the subjects after the exercise and diet regime were probably related to the higher plasma insulin concentrations. Bergström and co-workers (1967) reported a slight increase in blood glucose after carrying out a similar investigation in untrained subjects but did not measure insulin in the blood. However, the subjects in the Scandinavian study were taking a standardised diet, whilst those of the present study were at liberty to choose their own food within the confines of the broad outline given them at the start. It is a possibility that the higher resting levels of insulin are related to the slightly greater food intake during the second part of the diet, when the runners ate a high proportion of carbohydrate.

An increase in the value of blood glucose concentration has been noted in previous investigations of the metabolic changes during strenuous exercise (see Chapters 1,2,3 & 4). Such changes have also been reported by a number of other workers (Hermansen et al., 1970; Wahren et al., 1971). The greater rise in the glucose levels in the post-diet situation, although from a lower resting value, probably reflects the greater stores of liver glycogen (Hultman & Nilsson, 1971) and a decreased uptake of glucose by skeletal muscle, which was presumably glycogen-rich.

The greater blood concentrations of lactate and pyruvate in the runners after the glycogen-enhancing regime were similar

to the increases reported by Bergström et al. (1967). The increases probably reflect the greater reliance of the working muscle on enhanced glycogen stores and the production of energy by glycolysis. The lower lactate-pyruvate ratio during the second run indicates that despite the greater reliance on glycogen for fuel the runners were still able to remove the products of glycolysis by odixation.

The lower blood concentrations of glycerol and FFA at rest and throughout the period of exercise during the second investigation are capable of explanation on the basis of suppression of lipolysis by the elevated levels of insulin and possibly by the greater rise in glucose. There was also a smaller rise of HGH, a known lipolytic agent. It would be difficult to imagine how glycogen-rich muscle could signal to fat depots at rest but the increased production of lactate during exercise would decrease lipolysis and increase the rate of re-esterification of fatty acids (Fredholm, 1970;Fredholm, 1972; Issekutz & Miller, 1962).

Since fatty acids are apparently taken up by the muscle tissue according to the arterial concentration, it seems that the energy supply to the working muscle in the situation described above would be principally from intramuscular glycogen. However, the possibility remains that the exercise and diet regime may increase the intramuscular stores of lipid, which have recently been shown to contribute to the supply of fuel to working muscle (Fröberg, 1971).

The higher levels of blood ketone-bodies in the period of running during the post-diet investigation may be related either to a decreased peripheral uptake, perhaps by muscle, or to the

i ncreased production of ketone-bodies during exercise as a result of decreased redox-potential after carbohydrate oxidation. It is unlikely that the change in the concentration of the blood ketone-bodies affected the total energy economy of the muscle since the concentration of ketone-bodies is low during exercise compared to levels of other fuels.

The abolition of the development of "post-exercise ketosis" in the post-diet situation can not simply be related to the lower degree of fat mobilisation observed. The concentration of plasma FFA, although lower after the diet, nevertheless rose by about 25% compared to a rise of about 33% in the pre-diet run but the rise in the blood ketone-bodies was 300% at 105 min after exercise, compared with nearly 1200% after the glycogen enhancing regime. The most likely explanation of this difference is related to the higher levels of carbohydrate flux through the Kreb's cycle and the activation of acetyl CoA metabolism via citrate. The difference might also be the result of increased re-esterification of plasma FFA as a result of the higher lactate concentration but against this is to be put the possibly smaller degree of insulin induced re-esterification in the post-exercise period since the insulin levels continued to fall after the second run.

The smaller response of growth hormone to exercise after the glycogen enhancing regime is probably due to higher levels of insulin and possibly also of lactate and pyruvate during exercise. A further possibility is that the exercise-induced rise in protein catabolism is less after a high-carbohydrate diet, thus causing a smaller amino acid mediated release of HGH.

The plasma concentrations of immunoreactive insulin were much greater throughout the period of exercise after the glycogen enhancing regime; the fall in the levelwith exercise was also less. The higher intake of carbohydrate before the second run can explain the increased insulin concentration but the smaller observed fall in the insulin values is a new finding that is not explicable on the basis of the difference in the diet alone. Whether the differences are due to changes in the rate of secretion or uptake is unknown and the present results do provide information on this point.

Pruett (1970a) showed that the exercise-induced changes in insulin concentration were not different when the exercising subjects were investigated after fat, protein and carbohydrate rich diets. However, those studies were carried out in the post-absorptive state and there was no attempt to boost the levels of glycogen after previous exercise. The increased resting levels of insulin may have also resulted in a greater transport of glucose into muscle cells during the high carbohydrate diet.

The changes in the levels of the plasma IRI after exercise provide some evidence as to the nature of the normal stimulus to insulin at this time. Under normal circumstances insulin levels rise after strenuous exercise and this rise has been related (Chapters 2,3, 4 & 9) to high blood concentrations of fatty acids and of ketone-bodies. In the post-exercise period, after the glycogen-enhancing regime, there was little development of ketosis and insulin concentration remained lower than in the pre-diet situation when there was considerable ketosis. This is further evidence that the stimulus to increased release of

insulin after exercise is the rise of the level of ketone-bodies in the blood.

5.5 SUMMARY

1. Six long-distance runners were studied during and after a regime of exercise and diet to increase muscle glycogen content. Metabolic and hormonal changes were investigated during and after a 90 min run under normal conditions and again after the glycogen-enhancing regime.

2. Blood concentrations of glucose, lactate and pyruvate were greater during the second run. Blood glycerol and plasma FFA were lower. Blood ketone-body concentrations were higher during exercise and lower afterwards on the second occasion.

3. Plasma insulin levels were higher and fell less during the second run. Plasma growth hormone levels rose less during the second run.

4. The results are consistent with an increase in the proportion of carbohydrate used during exercise after an increase in muscle glycogen. The supply and utilisation of circulating FFA is apparently decreased. These changes may be partly related to an increase of plasma insulin and decrease in growth hormone during exercise.

CHAPTER 6

A STUDY OF PATIENTS SUFFERING FROM INAPPROPRIATE FATIGUE DURING EXERCISE ASSOCIATED WITH HIGH BLOOD LACTATE CONCENTRATIONS

6.1 INTRODUCTION

This chapter deals with the metabolic changes during exercise in a group of patients who were referred for a neurological investigation of possible organic muscle disease. All of the patients had complained of fatigue and muscular paid of several years duration and had attended various medical departments. No abnormalities had previously been found and several of the patients had been referred for a psychiatric opinion. No serious psychiatric illness was discovered.

Neurological assessment provided no clinical evidence of a myopathy and routine biochemical tests and muscle biopsies were normal.

Since these patients complained of an inability to carry out mild sustained exercise without fatigue it was decided to investigate the metabolic changes occurring during and after a period of moderate exercise.

6.2 PATIENTS

Six patients were studied. Details of the patients and their previous investigation are given in Table 6:i. The investigation was explained to the patients and their consent obtained. Each subject exercised on an electrically braked bicycle ergometer (Elema-Schönander) for up to 30 min at work loads of between 500 (1 patient) and 600 kpm/min. Venous blood samples were taken by means of an indwelling catheter at rest and at intervals during and after the period of exercise. Heart rate was recorded using an e.c.g. The results were compared with observations taken from previous investigations of normal

TABLE 6.i DETAILS OF PATIENTS AND PREVIOUS INVESTIGATIONS

PATIENTS					INVESTIGATIONS
	AGE	SEX	LENGTH OF HISTORY (YEARS)	PSYCHIATRIC REFERRAL	
J.R.	37	M	8	NO	General: E.C.G., X-Ray
M.F.	27	M	4	NO	Blood: Electrolytes, Urea pO_2 pCO_2 Enzymes Liver Function tests
T.Y.	26	M	5	NO	
A.L.	19	M	4	YES	
W.H.	34	F	12	YES	
L.G.	19	F	5	NO	Muscle Biopsy: Histology Histochemistry Electron Microscopy
					ALL NORMAL

volunteers. Blood samples were treated as described in Appendix I and analyses carried out for the estimation of glucose, lactate, pyruvate, glycerol, plasma free fatty acids and ketone bodies. Plasma insulin and growth hormone were estimated in three of the patients (J.R., T.Y. & W.H.). Four of the patients were investigated on subsequent occasions and the observations made compared with their current symptoms. Respiratory gas analysis was carried out by Dr. Sheila Jennett on one patient during a subsequent investigation.

6.3 RESULTS

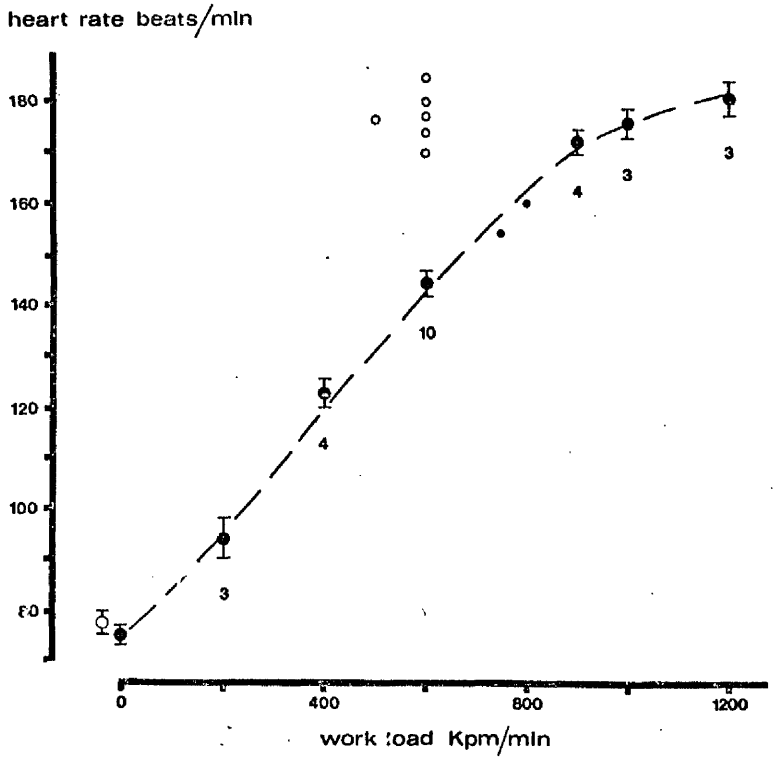
(a) Heart rate at rest and during exercise: (Fig. 6:i) The patients had resting heart rates of 78 $\pm$ 4 beats/min (mean $\pm$ SEM) compared to a figure for twelve controls of 76.5 $\pm$ 4. However, the patients showed a much greater rise during exercise than the controls reaching a maximum heart rate of between 168 and 185 at the end of the exercise period. This was outside the range of the heart rates in the normal controls for the same work load. The rate of recovery in the patients after exercise was also less than for the controls: at 5 min after the end of exercise the patients had heart rates of 126 $\pm$ 12 beats/min whilst the controls had fallen to 102 $\pm$ 8 beats/min (means $\pm$ SEM).

(b) Metabolites in the blood: (Figs.6:ii; 6:iii; 6:iv; 6:v) All metabolites at rest were within the normal limits and there was a considerable overlap between the values in the controls and the patients. The changes in the blood levels of glucose, plasma FFA and ketone-bodies during the period of the exercise test were similar in the patients and the controls (Table 6:ii).

Changes in the blood concentrations of lactate and pyruvate were, however, much different during the period of the exercise in

Fig. 6:i Heart rate (beats/min) versus work load (kpm/min) for normal control subjects (means $\pm$ SEM, ⊛) and six patients (individual values, o) suffering from fatigue with exercise.

Fig. 6:ii Blood lactate (μmol/ml) at rest, maximum achieved during exercise and at 5 min and 15 min post-exercise in ten normal control subjects and six patients suffering from fatigue with exercise (means $\pm$ SEM).


heart rate beats/min
180
160
140
120
100
80
0
400
800
1200
work load Kpm/min
3
4
10
4
3
3

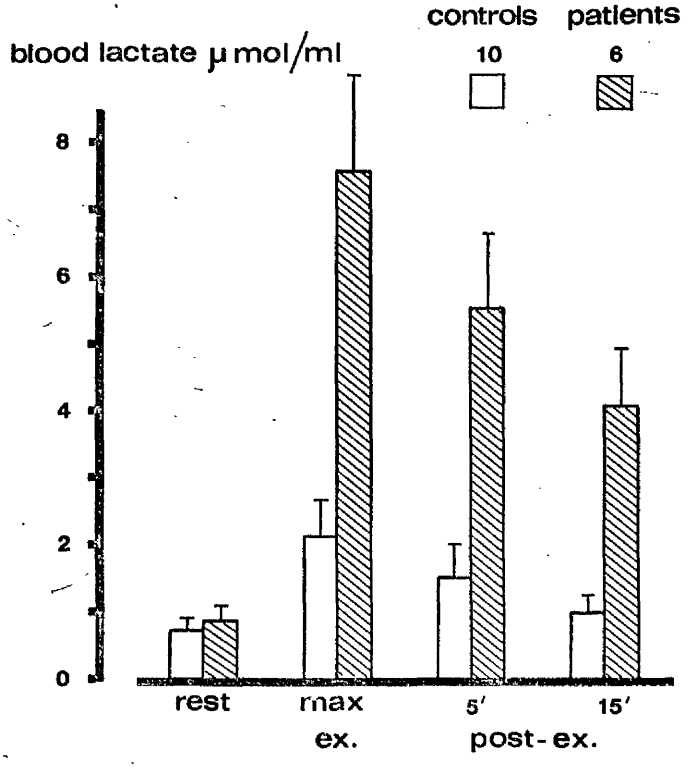
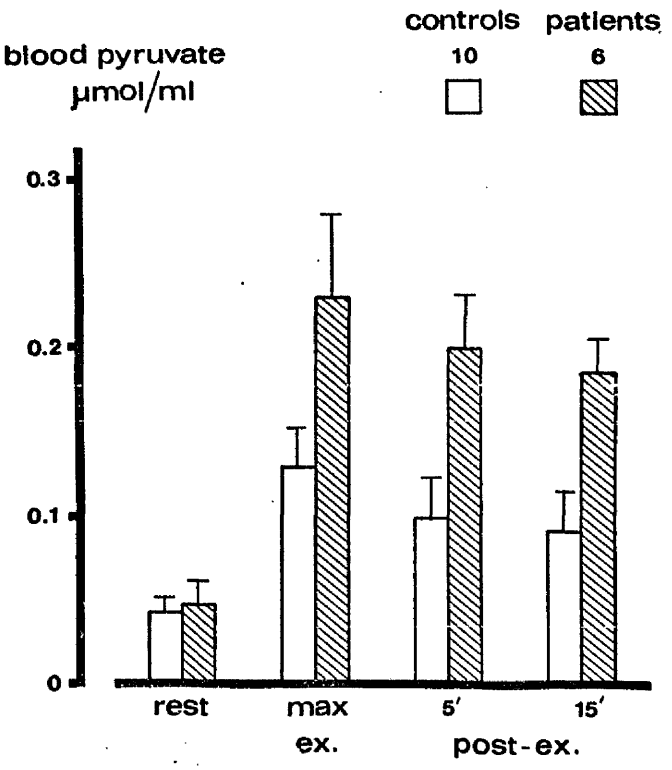

blood lactate µ mol/ml
controls
10
patients
6
8
6
4
2
0
rest
max ex.
5'
15'
post-ex.

Fig. 6:iii Blood pyruvate (μmol/ml) at rest, maximum achieved during exercise and at 5 min and 15 min post-exercise in ten normal control subjects and six patients suffering from fatigue with exercise (means ± SEM).

Fig. 6:iv Maximum blood lactate concentration (μmol/ml) during exercise versus work load for normal control subjects (means ± SEM o) and six patients suffering from fatigue with exercise (individual values o).


controls
patients
10
6
blood pyruvate
µmol/ml
0.3
0.2
0.1
0
rest
max
ex.
5'
15'
post-ex.

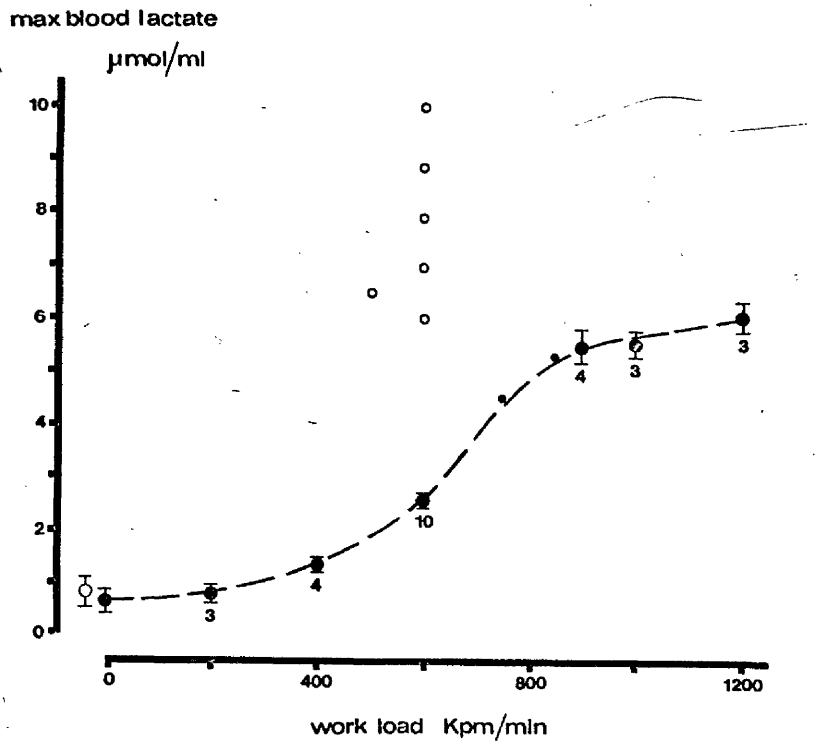

max blood lactate
µmol/ml
10
8
6
4
2
0
3
4
10
4
3
3
0
400
800
1200
work load Kpm/min

Fig. 6:v Blood ketone-bodies (μmol/ml) at rest, maximum achieved during exercise and at 5 min and 15 min post-exercise in ten normal control subjects and six patients suffering from fatigue with exercise (means $\pm$ SEM).

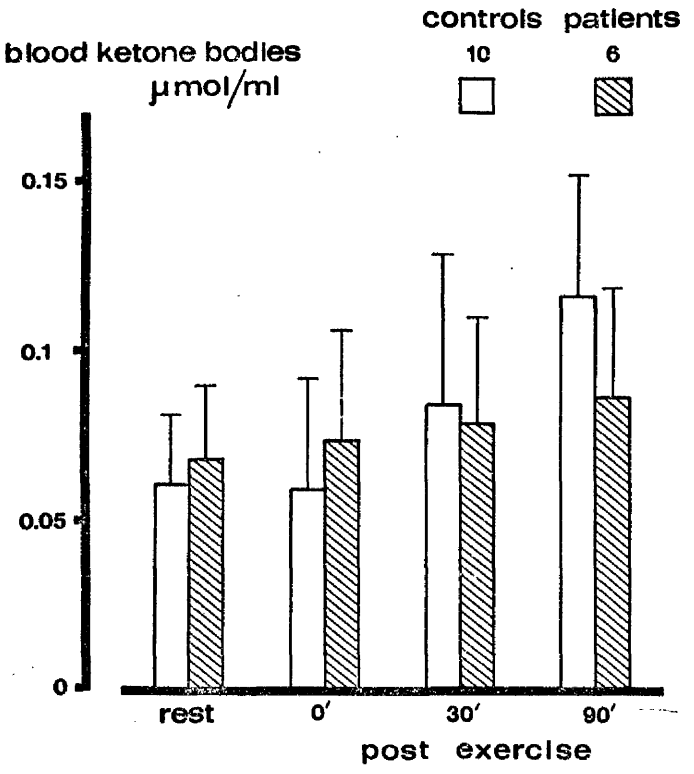

controls patients
10 6
blood ketone bodies
µmol/ml
0.15
0.1
0.05
0
rest
0'
30'
90'
post exercise

TABLE 6.ii BLOOD GLUCOSE (mg/100 ml) PLASMA FFA (μequiv/ml) AND BLOOD KETONE BODIES (μmol/ml) DURING EXERCISE IN PATIENTS AND CONTROLS. ALL VALUES MEANS ± SEM

Time(Mins)	REST		5		10		20		30	
	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls	Patients
Glucose	84.5 ±6.2	88.1 ±7.0	83.6 ±8.1	89.6 ±9.7	91.2 ±5.2	90.4 ±7.6	89.1 ±10.4	101 ±14.6	94.3 ±8.5	87.5 ±6.3
FFA	0.825 ±0.057	0.864 ±0.062	0.774 ±0.071	0.803 ±0.101	0.785 ±0.094	0.821 ±0.078	0.897 ±0.112	0.838 ±0.103	1.08 ±0.103	1.12 ±0.114
Ketone Bodies	0.078 ±0.018	0.091 ±0.035	0.072 ±0.074	0.082 ±0.032	0.081 ±0.018	0.088 ±0.032	0.007 ±0.009	0.092 ±0.041	0.091 ±0.027	0.076 ±0.010

the patients. Lactate concentrations rose much faster in the patients than in the controls so that at the maximum value achieved during exercise the values were 7.85 ± 1.21 μmol/ml (mean ± SEM) in the patients compared to 2.55 ± 0.13 μmol/ml in the controls. The blood lactate concentrations in the patients were higher even than levels which might have been predicted on the basis of the elevated heart rate. That is, the relative work load for the controls at heart rates of 168-184 would have been about 1200 kpm/min but the mean lactate concentration at this work load was only 6.02 ± 0.6 μmol/ml for the three controls compared to the maximum in the patients of 7.85 μmol/ml. Changes in the concentration of pyruvate during exercise in the patients mirrored the changes observed in the lactate values. The maximum values attained during exercise were higher than the values seen in the controls, either on the basis of absolute workload or on the basis of the work load appropriate to the heart rate achieved.

The changes in the blood concentrations of lactate and pyruvate in the patients after the end of the exercise were unremarkable; that is, despite the higher levels achieved during the period of the exercise, the rate of recovery was similar to that observed in the controls.

The lactate-pyruvate ratio was within the normal range at all times in the patients though it was slightly elevated.

The changes in the levels of the ketone-bodies and of their ratios to each other were similar in the patients to those observed in the controls after the end of exercise.

TABLE 6.iii PLASMA IRI (μunits/ml) AND HGH (μunits/ml) IN 3 PATIENTS AND 8 CONTROLS. ALL VALUES MEANS

		EXERCISE (600 kpm/min)				POST EXERCISE	
TIME (MINS)		REST	5	15	30	+15	+30
PLASMA IRI	Controls	10.2	9.7	7.5	8.1	10.3	12.2
	Patients	9.6	9.4	10.1	7.9	11.3	11.8
PLASMA HGH	Controls	8.1	10.8	15.6	22.1	17.2	12.0
	Patients	8.7	11.5	13.7	21.8	18.1	10.5

TABLE 6.iv BLOOD LACTATE IN 4 PATIENTS EXAMINED ON MORE THAN ONE OCCASION

Patient	Blood Lactate (μmol/ml)				Comments
	Rest	Max during ex	5 min Post-ex	15 min Post-ex	
A.L. (1)	0.49	10.80	9.12	8.97	Severe Fatigue: Collapse on 2nd occasion
A.L. (2)	0.62	9.41	7.36	6.38	
J.R. (1)	0.82	9.71	6.36	4.32	Progressive improvement
J.R. (2)	0.62	9.42	7.41	5.18	
J.R. (3)	0.77	6.10	5.41	3.48	
W.H. (1)	0.51	8.12	8.01	6.87	Very tired.
W.H. (2)	0.64	7.90	6.53	5.74	No change
T.Y. (1)	0.78	6.52	4.36	2.87	Less fatigue. Managed to go on ski-ing holiday
T.Y. (2)	0.81	4.10	3.00	1.02	

(c) Hormone changes with exercise: The resting levels of insulin and human growth hormone and the changes as a result of exercise were similar in the three patients in whom they were examined to those in a group of eight control subjects, for whom these hormones were examined. (see Table 6:iii). The changes in the blood levels of cortisol in the single patient in whom it was examined were within the range of changes observed in a group of normal controls and athletes (see Chapter 4).

(d) Results of subsequent tests: Table 6:iv shows the values of the maximum lactate concentration achieved during exercise for the three patients who were investigated on more than one occasion. Two of the patients reported no change in their symptoms (A.L. & W.H.) although the maximum lactate concentration was slightly lower on the occasion of the second test. The two other patients (J.R. & T.Y.) reported feeling less fatigued than previously and said that they were managing to take more exercise. In both of these patients the levels of lactate and pyruvate were still high compared with control values but they were considerably lower than on the previous tests. In one of these patients (T.Y.) the ventilation and the oxygen consumption during exercise were 52 and 2.8 l/min respectively. These figures were obtained during work at 600 kpm/min but were appropriate to a work load of about 1400 kpm/min in a normally trained subject (Dr. Sheila Jennett, personal communication).

6.4 DISCUSSION

The results of the exercise tests carried out by the patients demonstrate that their work capacity was very much

lower than that of normal controls of the same age and weight. In addition, their metabolic response to the exercise in terms of the changes in blood concentrations of lactate and pyruvate was inappropriate either to the work performed or to the relative work load predicted from the heart rate changes.

The source of the lactate and the pyruvate in the blood was probably working muscle having been produced by breakdown of stored glycogen. Since there was no difference in the levels of blood glucose between the patients and the controls it is unlikely that glycolysis from this source contributed to the high lactate and pyruvate concentrations. The lactate-pyruvate ratios in the patients were within the range which has been observed before in normal subjects and this suggests that tissue hypoxia did not cause the production of lactic acid. Another indication of the normal nature of the tissue redox state was that the ratios of 3-hydroxybutyrate-acetoacetate were also within normal limits. This ratio gives some indication of the redox state of mitochondria, as the lactate-pyruvate does of the cytoplasm of cells. The rate of disappearance of the lactate and pyruvate was similar in the two groups, indicating that the liver was handling these metabolites equally well.

Although there appeared to be a large increase in the flux of carbohydrate broken down within the muscle for fuel during exercise in the patients there was no corresponding increase in the supply of plasma FFA. This might have been a result of the suppression of lipolysis in the patients by the high levels of lactate (Fredholm, 1970: Issekutz & Miller, 1962). Indeed the lactate induced suppression of

lipolysis which might occur in this situation would increase the energetic dependence of the muscle upon glycolysis for fuel. Similarly, there was no build up of the blood concentrations of ketones in the patients after the end of the exercise period. The development of post-exercise ketosis has previously been associated with athletically unfit or untrained persons (Johnson et al., 1969; see Chapters 1-4) and its absence in these patients suggests that they were not simply unfit in that sense.

The patients described above have been shown to respond abnormally to a standard exercise test and show a pattern of low work capacity, high exercise heart rate, high lactic acid levels and inappropriate fatigue during exercise. One explanation which was suggested during their clinical examination was that their symptoms were similar to those of Da Costa's syndrome. However, none of these patients showed abnormal e.c.g. and none suffered chest pain or palpitations. Holmgren has described a condition which he has called vasoregularoty asthenia (Holmgren, Jonsson, Levander, Linderholm, Sjöstrand & Strőm, 1957). This is characterised by fatigue, a hyperkinetic circulation with marked orthostatic increase of heart rate and decreased a-v differences in blood oxygen content. However, his patients had lactate levels in the normal range. The patients described in the present chapter showed little signs of tissue hypoxia (i.e. normal L/P, 3HB/AcAc ratios) even though they had elevated lactates. Also, in the single patient examined, the oxygen consumption was in the range of normal to high. These patients do not, therefore, fit into Holmgren's syndrome although there is a superficial resemblance.

It is possible that these patients had an excessive sympathetic response to exercise. The urinary catecholamine excretion was, however, normal in two patients in whom it was measured, (A.L. & J.R.) although the blood levels of the catecholamines were not measured during exercise. Insulin levels, which are responsive to catecholamines, were also normal during exercise. However, it is also possible that the patients exhibited a greater metabolic and cardiovascular response to increases in the secretion of endogenous catecholamines. Sutton and co-workers have recently suggested an explanation of the so-called "hyperkinetic heart syndrome" in terms of hyper-responsiveness of beta-receptors in the cardiovascular system (Sutton, Seldon & Gunning, 1972). If such a hyper-responsiveness can also occur in the stimulation of muscle glycolysis then the overproduction of lactate and the symptoms of the patients described above might be e xplained.

Although the ultimate causes of the patients' illness remain obscure, it has been established that they do not suffer from a primary muscle disease nor an organic disorder of the heart and circulatory system. Similarly, there was no evidence of psychiatric disorders. However, there is considerable evidence of a disturbance of muscle carbohydrate metabolism which results in the production of lactic acid during exercise in amounts large enough to be responsible for the symptoms of fatigue upon exercise, as described by the p atients.

6.5 SUMMARY

1. Six patients who complained of inappropriate fatigue and muscle pain were referred for neurological investigation of possible muscle disease. Previous examination in a number of medical departments had not disclosed any abnormalities.

2. Neurological assessment showed no clinical evidence of a myopathy and muscle biopsies were normal.

3. The patients were studied during and after moderate exercise on a bicycle ergometer and blood samples taken for analysis of metabolites (all) and insulin and growth hormone (3).

4. The patients showed very large increases in blood lactate and pyruvate concentrations but there was little evidence of fat metabolism during and after exercise.

5. It is concluded that these patients do show an abnormal metabolic response to exercise. There is an apparent disturbance of muscle carbohydrate metabolism which results in overproduction of lactate sufficient to account for the patients symptoms of fatigue.

CHAPTER 7

GROWTH HORMONE AND EXERCISE - I

T he effect of moderate exercise on blood metabolites in patients with hypopituitarism.

7.1 INTRODUCTION

Moderate to severe exercise for periods longer than 20 min causes lipolysis in normal subjects and there is a rise in the blood concentration of FFA and ketone-bodies. The increases become most marked in the period after exercise (see Chapter 1). The blood concentration of FFA and ketone-bodies is lower in trained athletes than in untrained subjects. (Johnson et al., 1969; Jennett et al., 1971) Increased physical training in normally fit individuals resulted in smaller increases in plasma FFA and ketone-bodies after exercise. (Chapter 2, 3 & 4).

Differences in hormonal regulation of metabolism between athletically trained individuals and normal subjects might contribute to the observed differences in fat metabolism. Exercise causes a rise in the blood levels of human growth hormone (Hunter, Fonseka & Passmore, 1965). There is a smaller rise during exercise by trained athletes compared with untrained subjects (Sutton et al., 1968; 1969; see also Chapters 2 and 3).

An opportunity to investigate the role of HGH during and after exercise is provided by studying the metabolic response to exercise in patients suffering from hypopituitarism, since HGH levels are low and respond poorly to common stimuli of growth hormone release (Nieman, Landon & Wynn, 1967).

7.2 METHODS

Six patients with hypopituitarism (17-63 years) and

eight normal, untrained subjects (19-63 years) were studied. The patients had clinical symptoms of pituitary tumour and the diagnosis of chromophobe adenoma was confirmed histologically after operation. Their particulars are given in Table 7:i. Four (A.McC., A.C., J.W., & J.S.) patients were investigated before operation for their tumour and were receiving no replacement therapy at that time. Two of the patients (J.McM. & A.M.) were investigated after operation when they were receiving cortisone acetate (12.5 mg b.d.) and thyroxine (0.1 mg daily) as replacement therapy.

All subjects had fasted for at least 12 hr and the investigations were carried out between 10.00 hr and 15.00 hr; the temperature in the laboratory was about 16°C. They exercised on a bicycle ergometer for 30 min at a steady rate and resistance, which was similar in each investigation. The work load was estimated from oxygen consumption in three subjects as 400 ± 100 kpm/min (Dr. Sheila Jennett). Blood samples (20 ml) were taken by venepuncture before exercise, at three ten min intervals during exercise and immediately after exercise and then at 30 min intervals for 1½ hr. Blood samples were treated as described in Appendix I and analysed for lactate and pyruvate, acetoacetate, 3-hydroxybutyrate, glucose and glycerol. Glucose was studied in three of the patients only (J.McM., A.M., & J.S.). A plasma sample was analysed for free fatty acids (FFA) and a serum sample was analysed for growth hormone. All analyses were carried out as described in Appendix I. Significance of difference was tested using the Mann-Whitney u non-parametric test for small samples.

TABLE 7:i

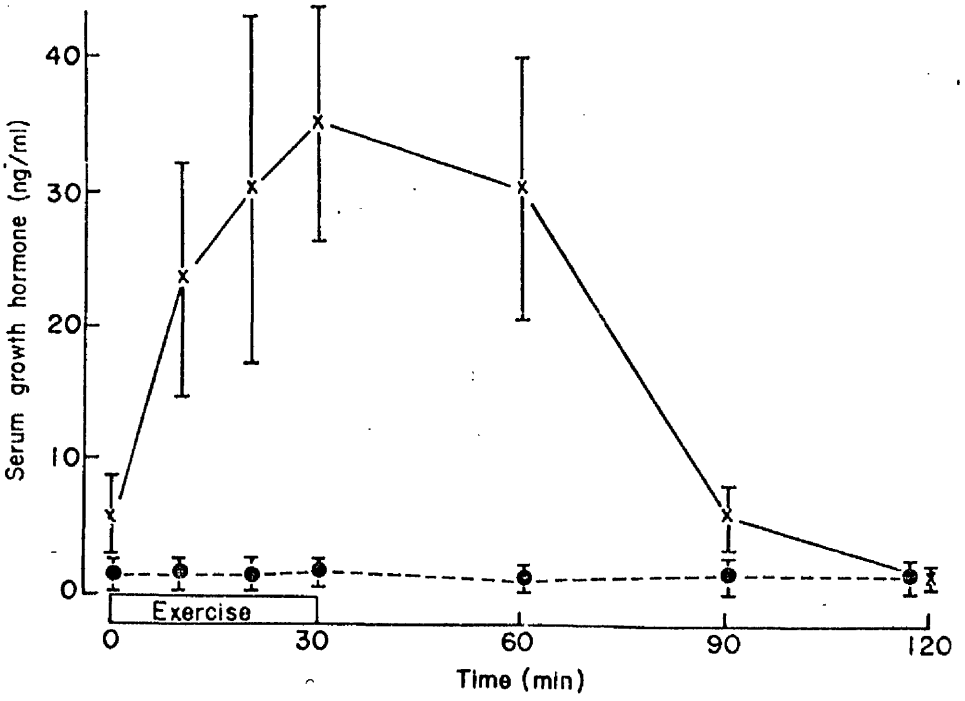
Details of eight controls and six hypopituitary patients

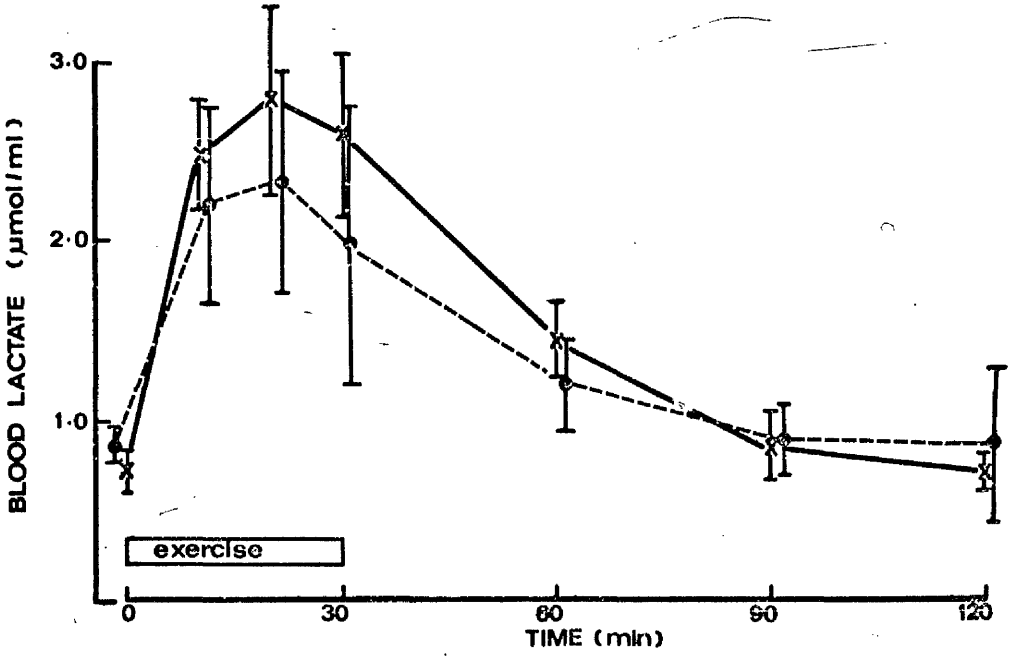
Controls	Sex	Age (Yrs)	Height (cm)	Weight (kg)	Reciprocal Ponderal Index ($ht/\sqrt[3]{wt}$)	Clinical & Histological Diagnosis
D.M.	M	19	170	66	42.07	-
F.C.	M	19	184	92	40.76	-
N.B.	M	20	185	76	43.67	-
J.H.	M	23	181	84	41.33	-
R.H.	M	54	158	56	41.32	Late onset epilepsy
R.McC.	F	54	157	48	43.01	Cervical spondylosis
M.McN.	F	59	157	60	42.59	-
J.B.	M	63	174	70	41.22	Meningioma
Mean		38.88	170.75	69.00	42.00	
SEM		±7.13	±4.30	±5.18	±0.36	
Patients						
A.McC.	M	17	157	49	42.90	
A.C.	F	38	158	89	39.20	
J.S.	F	49	175	81	40.45	Chromophobe Adenoma
J.McM.	M	56	169	94	37.42	
J.W.	M	61	175	78	42.90	
A.M.	F	63	152	80	35.28	
Mean		47.33	164.3	75.5	39.37	
SEM		±7.11	±4.1	±6.4	±1.11	

All patients except J.McM. and A.M. were investigated pre-operatively and were not receiving replacement therapy. J.McM and A.M. were investigated after operation and were receiving cortisone acetate (12.5 mg b.d.) and thyroxine (0.1 mg daily). The reciprocal ponderal index in the patients was significantly different from the controls ($P < 0.005$ by Mann-Whitney u Test).

Fig. 7:i Serum growth hormone ng/ml (mean $\pm$ 1 SEM) in 8 controls (x) and six hypopituitary patients (⊛) during and after 30 min exercise.

Fig. 7:ii Blood lactate levels μmol/ml (mean $\pm$ 1 SEM) in 8 controls (x) and six hypopituitary patients (⊛) during and after 30 min exercise.


Serum growth hormone (ng/ml)
40
30
20
10
0
Exercise
0
30
60
90
120
Time (min)


BLOOD LACTATE (μmol/ml)
3·0
2·0
1·0
exercise
0
30
60
90
120
TIME (min)

7.3 RESULTS

7.3.1 Subjects: The patients appeared fatter and shorter than the controls and their mean reciprocal ponderal index (RPI = ht ÷ 3 $\sqrt{wt}$) was greater than that of the controls (Table 7:i).

7.3.2 Growth Hormone and Blood Metabolite Changes:

(a) Serum growth hormone: (Fig. 7:i) Growth hormone rose in the control subjects during exercise and returned to resting levels in the subsequent 60-90 min. The patients had low resting levels of growth hormone and showed no increase with exercise, which confirmed their diagnosis.

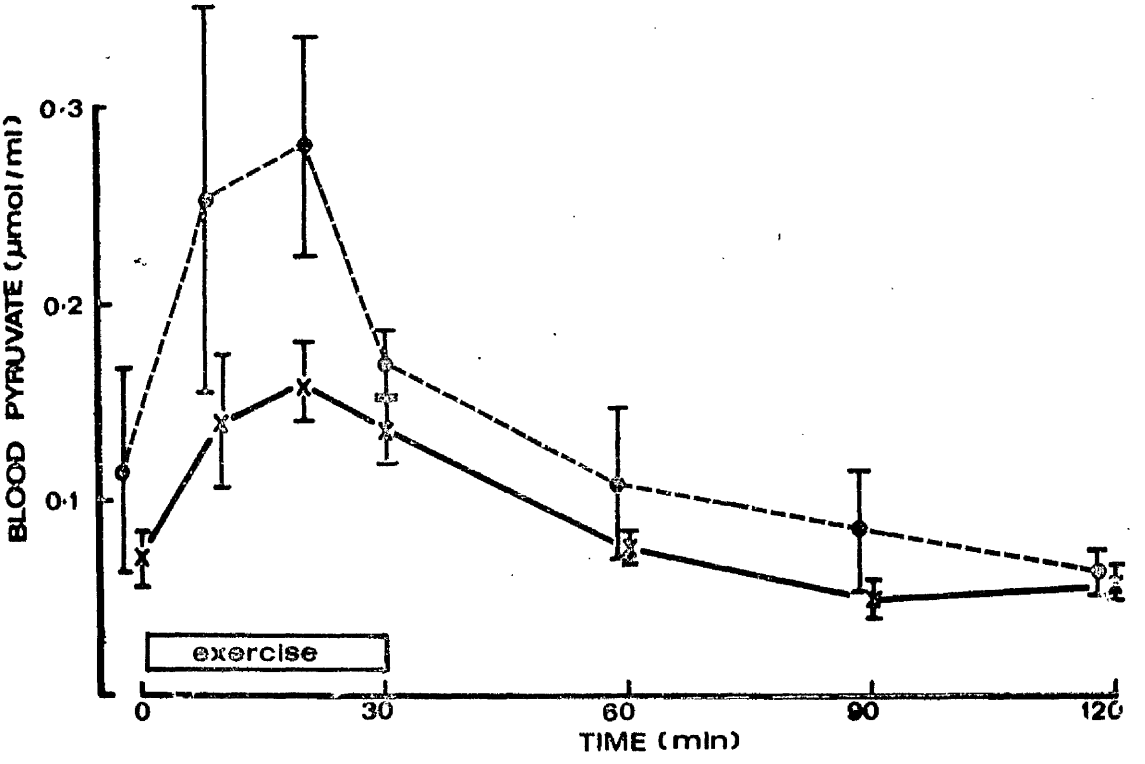
(b) Lactate: (Fig. 7:ii) Resting blood lactate levels were similar in both groups. During exercise the levels rose in a similar fashion in both controls and patients to a maximum after 20 min of exercise when there was no significant difference between the two groups ($P > 0.05$). The lactate concentration returned to resting levels by the end of the investigation.

(c) Pyruvate: (Fig. 7:iii) Resting blood pyruvate levels in controls and patients were not significantly different ($P > 0.05$). Exercise caused a marked rise which was greater in patients (150% increase at 20 min) than in controls (125% increase at 20 min.) The pyruvate concentrations then fell towards normal levels by the end of the experiment.

The lactate-pyruvate ratios (Fig. 7:iv) were higher in the controls during exercise and they reached a maximum

Fig. 7:iii Blood pyruvate levels μmol/ml (mean ± 1 SEM) in 8 controls (x) and six hypopituitary patients (⊗) during and after 30 min exercise.

Fig. 7:iv Lactate:pyruvate ratios (mean ± 1 SEM) in 8 controls (x) and 6 hypopituitary patients (⊗) during and after 30 min exercise.


BLOOD PYRUVATE (μmol/ml)
0·3
0·2
0·1
exercise
0
30
60
90
120
TIME (min)

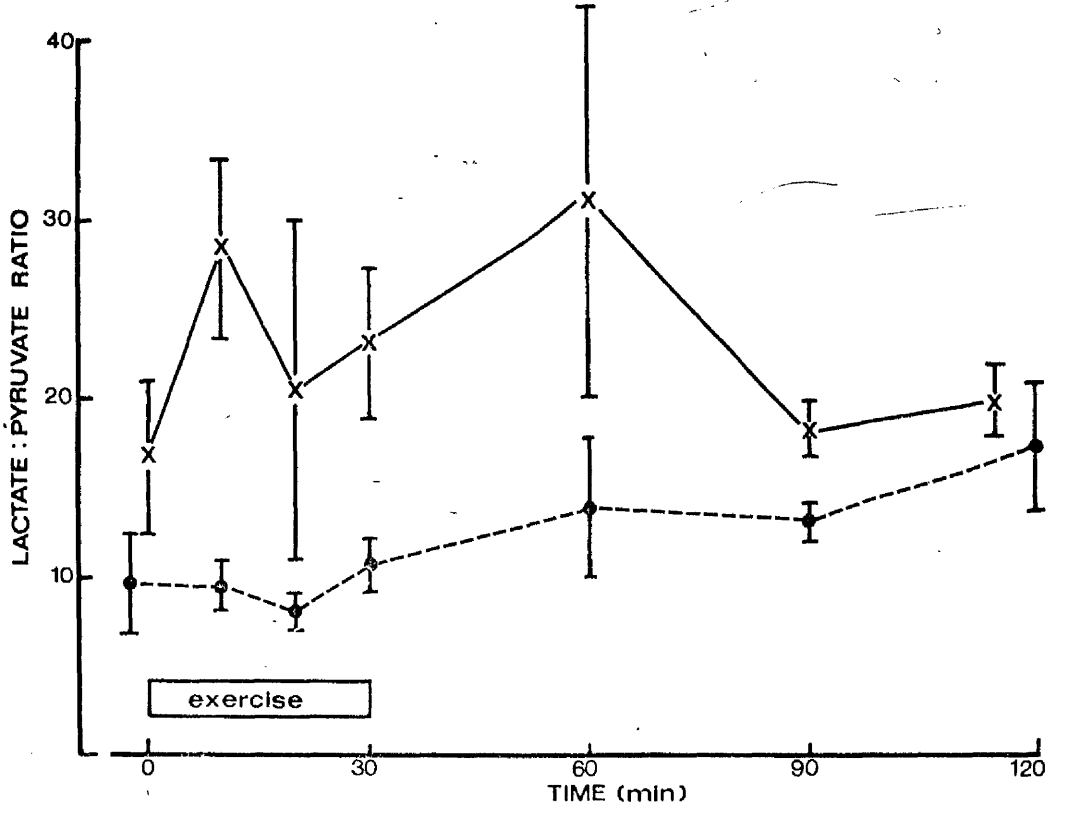
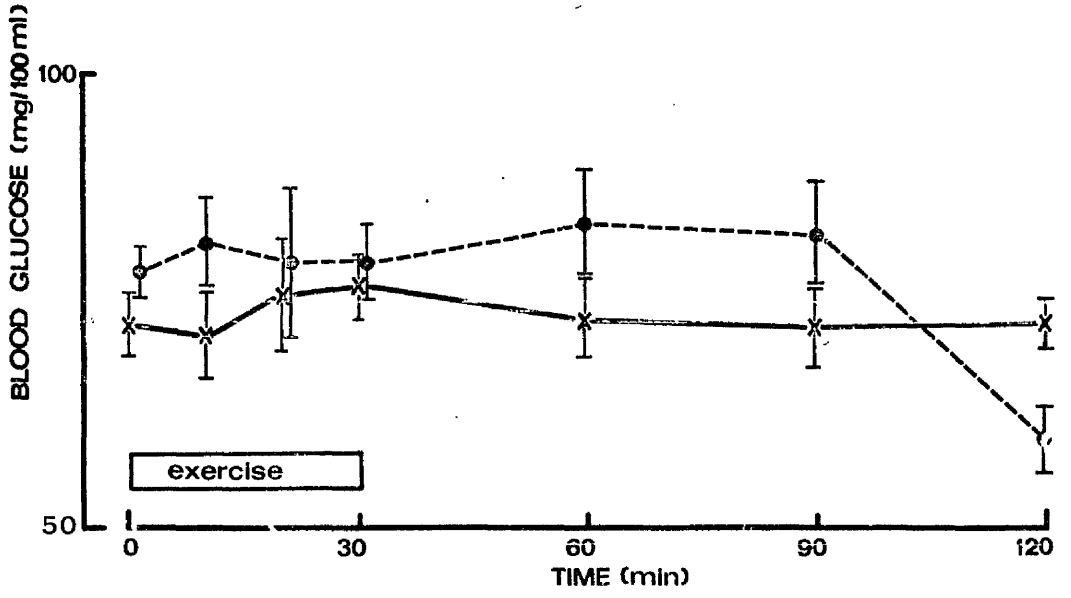

LACTATE : PYRUVATE RATIO
40
30
20
10
exercise
0
30
60
90
120
TIME (min)

Fig. 7:v Blood glucose levels mg/100 ml (mean $\pm$ 1 SEM) in 8 controls (x) and 3 hypopituitary patients (●) during and after 30 min exercise.

Fig. 7:vi Blood glycerol µmol/ml (mean $\pm$ 1 SEM) in 8 controls (x) and 6 hypopituitary patients (●) during and after 30 min exercise.


BLOOD GLUCOSE (mg/100 ml)
100
50
exercise
0
30
60
90
120
TIME (min)

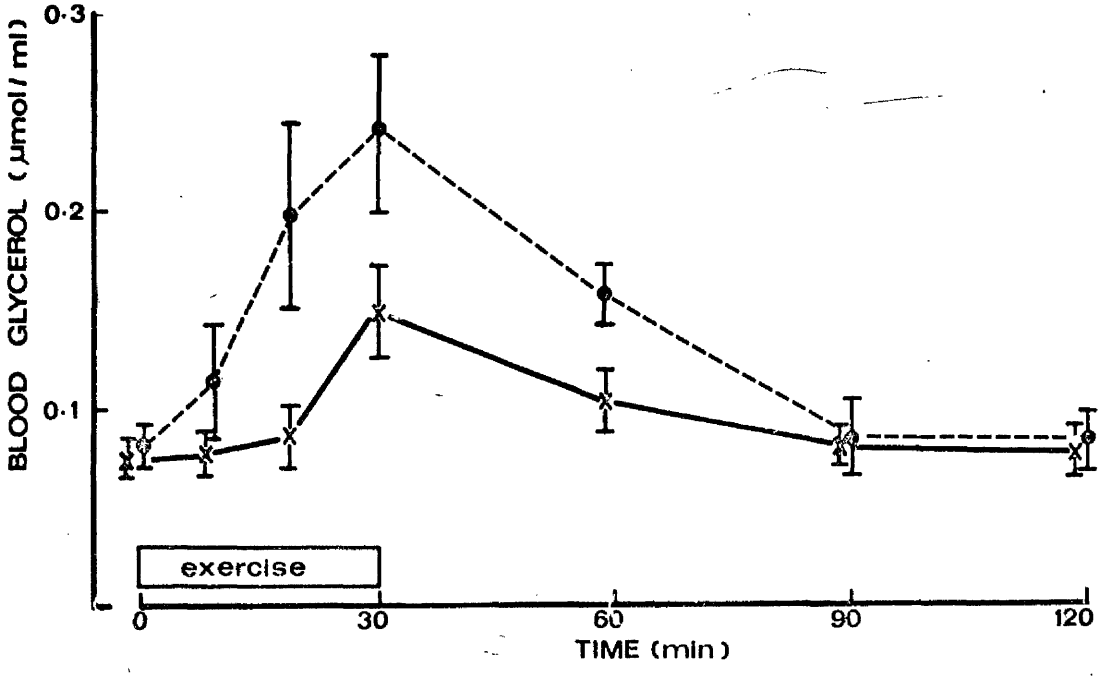
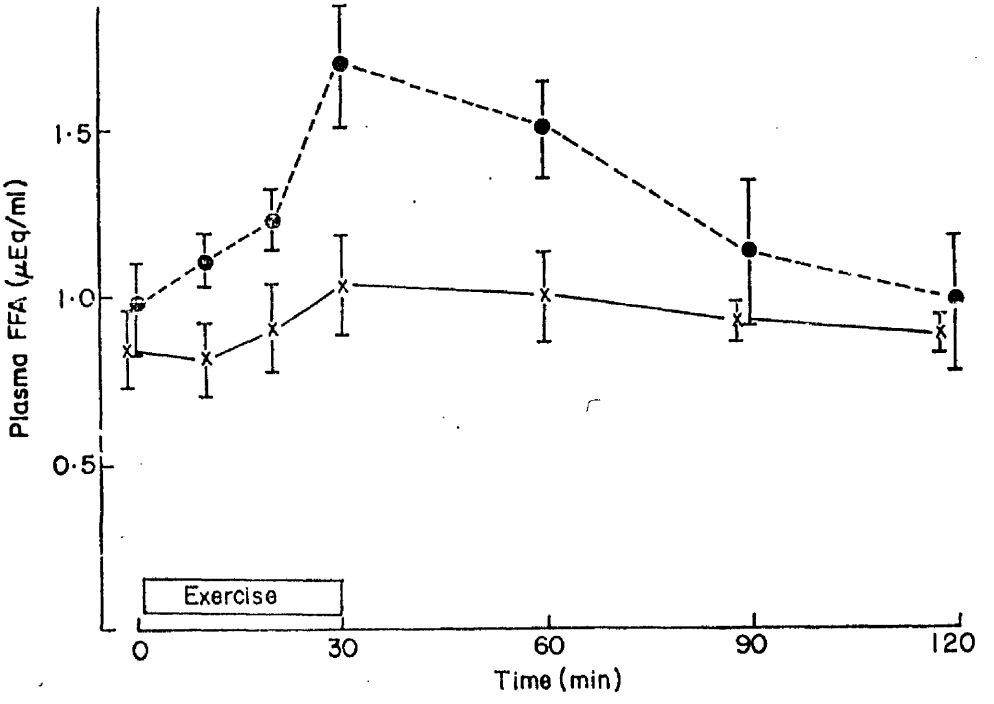
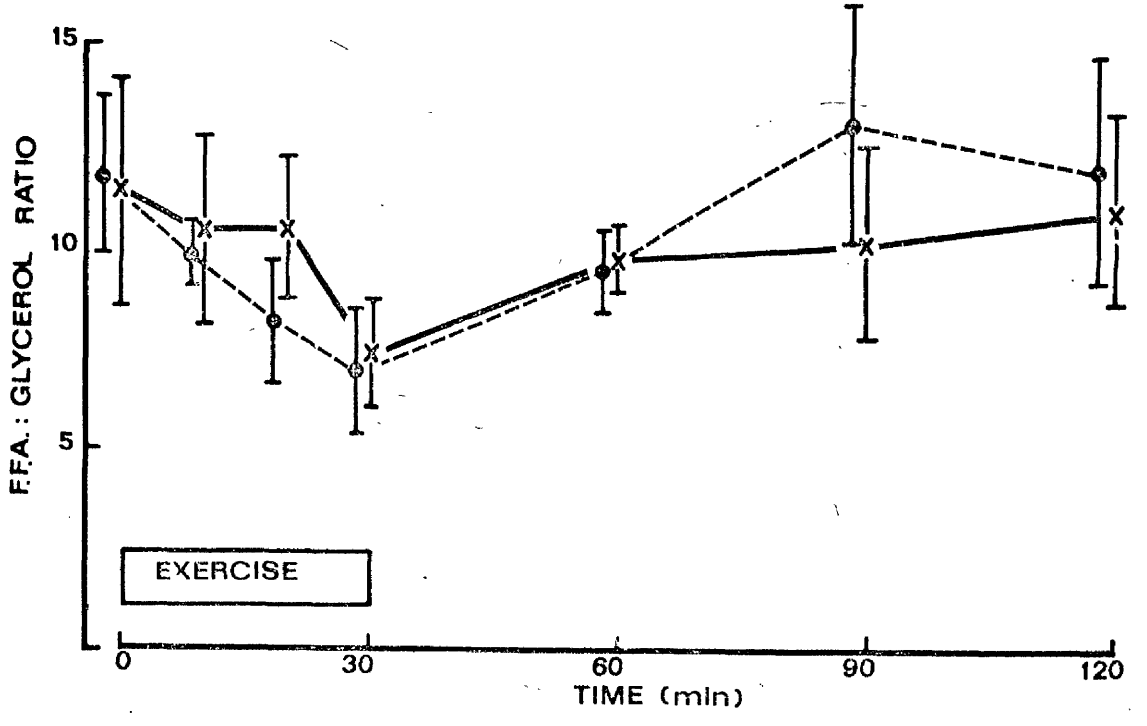

BLOOD GLYCEROL (μmol/ml)
0·3
0·2
0·1
exercise
0
30
60
90
120
TIME (min)

Fig. 7:vii Plasma free fatty acids, μ equiv/ml, (mean ± 1 SEM) in 8 controls (x) and 6 hypopituitary patients (●) during and after 30 min exercise.

Fig. 7:viii FFA:glycerol ratios (mean ± 1 SEM) in 8 controls (x) and 6 hypopituitary patients (●) during and after 30 min exercise.


Plasma FFA (μEq/ml)
1·5
1·0
0·5
Exercise
0
30
60
90
120
Time (min)


F.F.A.: GLYCEROL RATIO
15
10
5
EXERCISE
0
30
60
90
120
TIME (min)

30 min after exercise.

(d) Glucose: (Fig. 7:v) Blood glucose levels were slightly higher throughout in the three patients examined, except at 90 min following exercise when they were significantly depressed ($P < 0.05$).

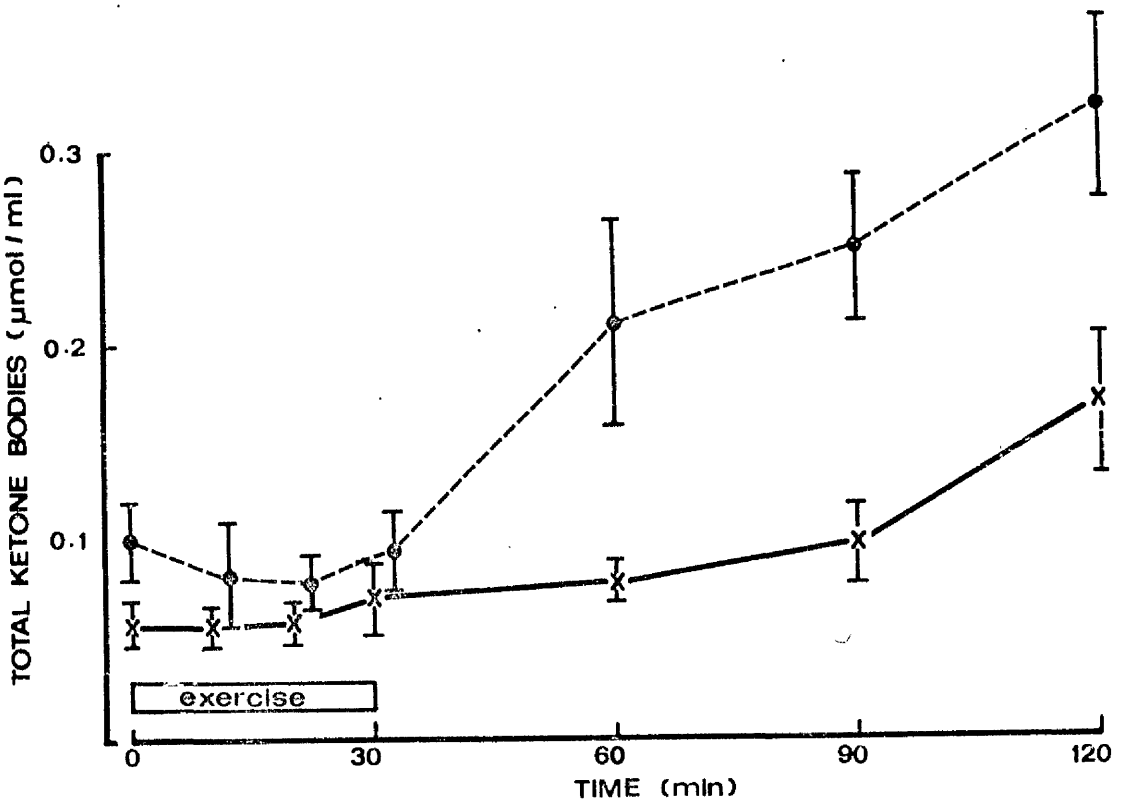
(e) Glycerol: (Fig. 7:vi) There was no significant difference in blood glycerol levels between the two groups ($P > 0.05$). The concentration rose during exercise and at the end of exercise it had increased nearly four times in the patients and doubled in the controls. Resting levels were achieved within 60 min of the end of exercise in both groups.

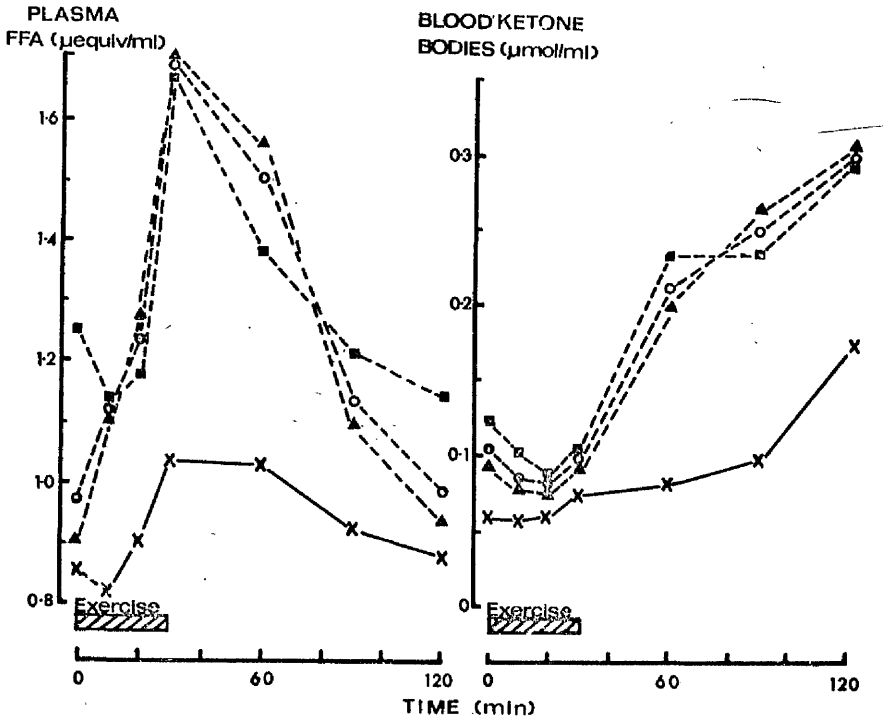
(f) FFA: (Fig. 7:vii) Plasma FFA levels were similar at rest in both groups. They rose by 74% in the patients and 22% in the controls by the end of exercise. The controls showed a small fall in FFA concentration 10 min after the beginning of exercise. In the 90 min following exercise FFA fell to resting levels in both groups. FFA-glycerol ratios are given in Fig. 7:viii.

(g) Ketone-Bodies: (Fig. 7:ix) Blood ketone-body concentrations (acetoacetate plus 3-hydroxybutyrate) were significantly higher at rest in the patients compared with controls ($P < 0.05$). Differences between values at rest and after 30 min of exercise were not significant in both groups ($P > 0.05$). After exercise the concentrations increased considerably and the levels were higher in the patients throughout the rest of the investigation.

Fig. 7:ix Total blood ketone bodies μmol/ml (mean ± 1 SEM) in 8 controls (x) and 6 hypopituitary patients (⊙) during and after 30 min exercise.

Fig. 7:x Plasma free fatty acids (μequiv/ml) and total ketone-bodies (μmol/ml) in 8 controls (x:means) and 6 hypopituitary patients (○: means). The results in the patients have also been shown in two groups: A. two patients on replacement therapy, 12.5 mg cortisone acetate b.d. and thyroxine 0.1 mg daily (⊡: means); B. four patients without replacement therapy (▲:means).


TOTAL KETONE BODIES (μmol / ml)
0.3
0·2
0·1
exercise
0
30
60
90
120
TIME (min)


PLASMA
FFA (μequiv/ml)
1·6
1·4
1·2
1·0
0·8
Exercise
0
60
120
BLOOD KETONE
BODIES (μmol/ml)
0·3
0·2
0·1
0
Exercise
0
60
120
TIME (min)

7.3.3 Effect of Replacement Therapy: (Fig. 7:x) The two patients who were receiving replacement therapy at the time of investigation showed changes similar to those in the four patients pre-operatively who were not receiving replacement therapy. There was no evidence to suggest that the replacement therapy exaggerated any differences during and after exercise between the control group and the four patients who were receiving no replacement therapy when investigated.

7.4 DISCUSSION

Exercise caused a rise in growth hormone in the normal subjects which was absent in the patients, thus confirming the diagnosis of pituitary insufficiency in the patients. Exertion is known to cause elevation of lactate and pyruvate levels in normal subjects and the concentrations of lactate were not significantly different in both groups during and after exercise. The pyruvate levels in the patients, however, were significantly higher towards the end of exercise. The depression of the lactate-pyruvate ratio of the patients suggests that oxidative mechanisms other than those of carbohydrate metabolism, such as those involved in fat catabolism, may have contributed more to the oxidative activity in the patients during exercise.

The patients showed a greater blood glucose concentration throughout, apart from the fall 90 min after exercise, and this may be further evidence for the greater reliance on fat for fuel during exercise

by the patients. This may also have been related to their relative obesity (Haar, van Riet, Thijssen & Schwarz, 1969).

At the end of exercise FFA and glycerol had increased more in the patients than in the controls despite the patients' low growth hormone levels. A similar change has been reported in two patients on replacement therapy by Basu, Passmore & Strong (1960). The higher levels of glycerol and FFA in the patients may reflect greater rates of fat mobilisation and utilisation (Winkler, Steele & Alszuler, 1969). The FFA-glycerol ratio, however, was similar in each group indicating equal rates of catabolism in both. Previous studies have shown similar changes in FFA in persons not in athletic training compared with athletes and, therefore, the differences in our subjects may be due to differences in athletic fitness. Such an explanation would require a greater fall in the FFA-glycerol ratio in the patients (Johnson et al., 1969) but this did not occur. Also, the lactate-pyruvate ratios (which are lower during exercise in athletically trained individuals compared to untrained subjects) were higher in the controls.

The control group showed a depression of FFA levels at 10 min after beginning exercise which has previously been reported during the first 10-20 min of exercise (Hartog, Havel, Copinschi, Earl & Ritchie, 1967; Harris, Bateman, Bayley, Donald, Gloster & Whitehead, 1968). Its absence in the patients is

unexplained but could be because the sampling times were widely spaced and the response had been missed in the patients, or they may have been capable of more rapid mobilisation of FFA.

Both patients and controls showed a rise in ketone-body concentration after exercise and the increase, as with FFA, was greater in the patients. A relatively low level of post-exercise ketosis is a characteristic of athletic training (Johnson et al., 1969) and, as suggested earlier, the patients might have been less fit. Nevertheless, there was little difference in lactate levels between the two groups, which might have been expected if fitness was the total explanation.

It has been suggested that growth hormone has a major role in the initial mobilisation of fat during exercise (Hunter et al., 1965; Rabinowitz, Klassen & Zierler, 1965; Winkler et al., 1969) but growth hormone release has been shown to occur after the increases of FFA and glycerol (Hartog et al., 1967). The rise in FFA in our patients also fails to support such a role for growth hormone. A rise in FFA has also been reported after starvation in hypophysectomized dogs (Goodman & Knobil, 1961), after exercise in two subjects with hypopituitarism receiving cortisone and thyroxine replacement therapy (Basu et al., 1960). It has also been shown that the rise in growth hormone is not dependent on a previous rise in FFA, as it occurred when fat mobilisation was blocked with nicotinic acid (Hartog et al., 1967). The possibility that growth

hormone is related to the rise in ketone-bodies after exercise is weakened further by the present results, as in the normal subjects growth hormone was falling after exercise when the ketone bodies increased. A rise in ketone-bodies after ingestion of acetoacetate also fails to cause a rise in growth hormone levels (Johnson, Sulaiman & Webster, 1972).

The rise of glycerol, FFA and ketones in the hypopituitary patients who were not receiving replacement therapy also suggests that the possible absence of other pituitary hormones with a direct or indirect adipokinetic effect (e.g. ACTH, TSH, MSH, arginine-vasopressin) does not necessarily inhibit exercise lipogenesis. This has been shown in the hypophysectomized rat (Gollinck, Soule, Taylor, Williams & Ianuzzo, 1970), in which lipolysis during exercise was only abolished by a combination of hypophysectomy and beta-blockade of adrenergic and noradrenergic induced lipolysis. The greater lipolysis in the patients is not readily explicable by increased adrenergic activity, however, since adipose tissue in monkeys may be demonstrated to be less sensitive to catecholamines after hypophysectomy (Goodman & Knobil, 1959).

Although the differences in FFA and ketone-bodies with exercise between patients and controls may be ascribed to differences in fitness, another possibility is that growth hormone depresses FFA levels after the initial mobilisation of fat with exercise.

This effect could be mediated through an increased re-esterification of FFA at the site of its release from adipose tissue as suggested by Winkler et al., (1969).

It can, nevertheless, be concluded that exercise causes changes in blood metabolites in hypopituitary patients of a similar degree to controls although the changes in FFA, glycerol and ketone-bodies tended to be more marked. Thus these changes can occur even when there is no change in the level of growth hormone.

7.5 SUMMARY

1. Six patients with panhypopituitarism and eight controls were studied before, during and after 30 min of moderate steady exercise on a bicycle ergometer. Venous blood samples were taken for estimation of growth hormone, pyruvate, lactate, glucose, FFA, glycerol and ketone-bodies.

2. Exercise caused a rise in serum growth hormone levels in control subjects and these fell to pre-exercise levels within 90 min. There was no change in the levels of the patients. The patients showed changes in blood metabolites resembling those of the controls. FFA levels were considerably elevated and post-exercise ketosis developed, these changes being more marked in the patients.

3. The differences in FFA and ketone-bodies between patients and controls during exercise may be due to the lower levels of fitness in the patients, or inhibition of

lipolysis stimulated by ketone-bodies might be impaired. A further explanation may be that growth hormone depresses FFA levels after initial mobilisation during exercise in normal subjects, but no such action can occur in patients with hypopituitarism.

4. It is concluded that growth hormone does not necessarily have a major role in fat mobilisation or in the production of post-exercise ketosis. Thus, the lower levels of ketosis observed in athletes are not due to their lower levels of growth hormone.

CHAPTER 8

FAT AND CARBOHYDRATE METABOLISM AT REST IN PATIENTS WITH HYPOPITUITARISM

8.1 INTRODUCTION

Hypopituitary patients show a flattened glucose tolerance curve and an impaired rise in FFA after oral glucose (Stamp, 1965). They are very insulin sensitive and, in contrast to patients who have suffered pancreatomy and who need 40 units of insulin per day, hypopituitary patients who become diabetic need as little as 2 units per day and severe ketosis may be controlled by 8 units (Di Raimondo, 1963, quoted by Evans, Briggs & Dixon, 1966 in Harris 1969). Insulin sensitivity appears to result in increased suppression of fat mobilisation during glucose tolerance tests in hypopituitary patients but nevertheless in the exercise investigation described in Chapter 7 lipolysis was greater in the patients than in the normal control subjects. Insulin rises after exercise in normal subjects and may promote re-esterification of FFA and suppression of ketone-body formation by liver (see Chapters 2, 3 and 4). Although there is no information available on insulin levels in hypopituitary patients after exercise it has been possible to examine the metabolic response of some of the patients to oral glucose and acetoacetate tests in which the normal hormonal and metabolic changes are known.

8.2 PROCEDURE

Three patients who had previously undergone the exercise test (A.M., J.McM. & J.S.) were subjected to a glucose tolerance test and an acetoacetate tolerance test on separate occasions. The subjects had fasted for at least 8 hr and remained at rest throughout the investigations, which were carried out in the morning.

In the case of the oral glucose tolerance test (OGTT) a venous blood sample was taken before ingestion of 50 g glucose powder in 250 ml of water. Blood samples were then taken at 30 min intervals for 180 min. The procedure was similar for the acetoacetate tolerance tests except that after ingestion of 200 ml of 0.4 M acetoacetate (Krebs & Eggleston, 1945) blood samples were taken at 40 min and then at 20 min intervals for a further 80 min. Blood samples were analysed for metabolites as previously described. The results were compared with those obtained from a number of subjects (3 male, 4 female, aged 22-58) who were not obese, had no evidence of metabolic or neurological disease and who had previously undergone similar tests. They were, however, not members of the control group who previously took part in the exercise investigation.

8.3 RESULTS OF GLUCOSE TOLERANCE TESTS

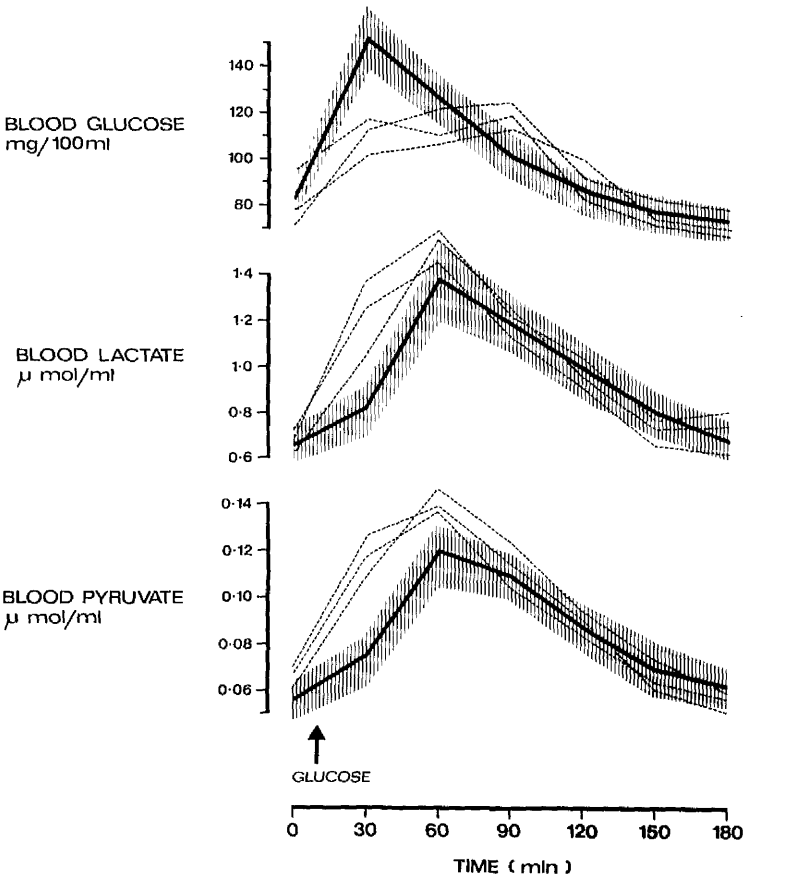
(a) Blood glucose, lactate and pyruvate: (Fig. 8:i) The resting levels of blood glucose were similar in each group. The glucose tolerance curves in the three patients were significantly ($P < 0.05$) lower than the mean of the control values at 30 and 60 min and greater at 90 min but the values at times from 120 to 180 min after ingestion of glucose were within the 95% range of mean control values.

Lactate and pyruvate concentrations were elevated at 30 and 60 min in the patients but thereafter the values were within the control limits.

(b) Plasma FFA, blood glycerol and ketone-bodies: (Fig. 8:ii) Resting levels of FFA, glycerol and ketone-bodies were similar in both groups. In both patients and controls

Fig. 8:i Changes in blood glucose (mg/100 ml), lactate (μmol/ml) and pyruvate (μmol/ml) before and after ingestion of 50 g glucose by 7 normal control subjects (means, solid line; hatching represents ± 2 SEM) and 3 hypopituitary patients. (broken line).

Fig. 8:ii Changes in blood glycerol (μmol/ml), plasma FFA (μequiv/ml) and blood ketone-bodies (μmol/ml) before and after ingestion of 50 g glucose by 7 normal control subjects (means, solid line; hatching represents ± 2 SEM) and 3 hypopituitary patients. (broken line).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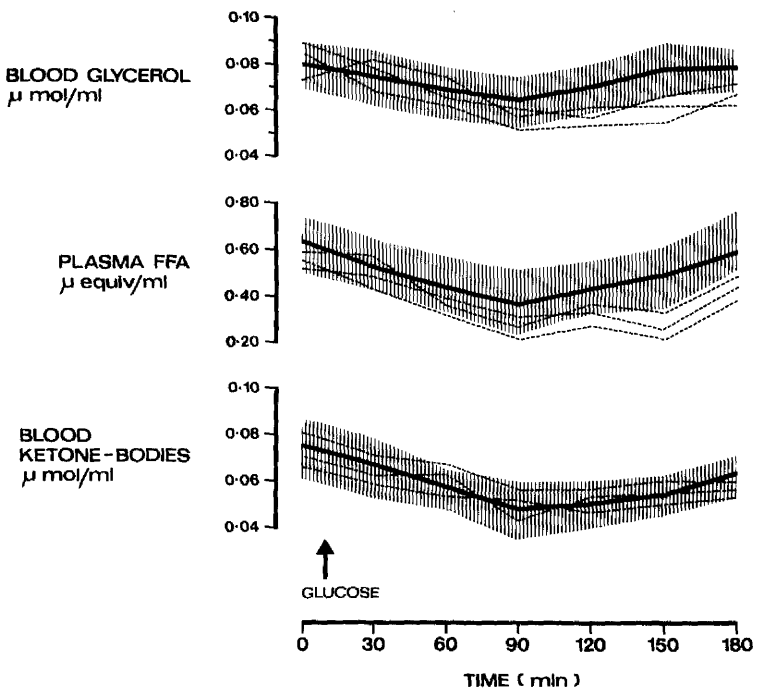
mg/100ml
140
120
100
80
BLOOD LACTATE
μ mol/ml
1·4
1·2
1·0
0·8
0·6
BLOOD PYRUVATE
μ mol/ml
0·14
0·12
0·10
0·08
0·06
GLUCOSE
0
30
60
90
120
150
180
TIME (min)


BLOOD GLYCEROL
μ mol/ml
0·10
0·08
0·06
0·04
PLASMA FFA
μ equiv/ml
0·80
0·60
0·40
0·20
BLOOD
KETONE-BODIES
μ mol/ml
0·10
0·08
0·06
0·04
GLUCOSE
0
30
60
90
120
150
180
TIME (min)

ingestion of 50 g glucose caused a depression in lipolysis and a fall in levels of the fat derived metabolites, but compared to the mean control values the fall was greater and persisted longer in the hypopituitary patients. However, only at 150 min were the concentrations of glycerol and FFA outside the range of control values although they were lower by more than 1 SEM at 120 and 180 min. The ketone-body concentrations were at no time further removed than 1 SEM from the mean of the control values.

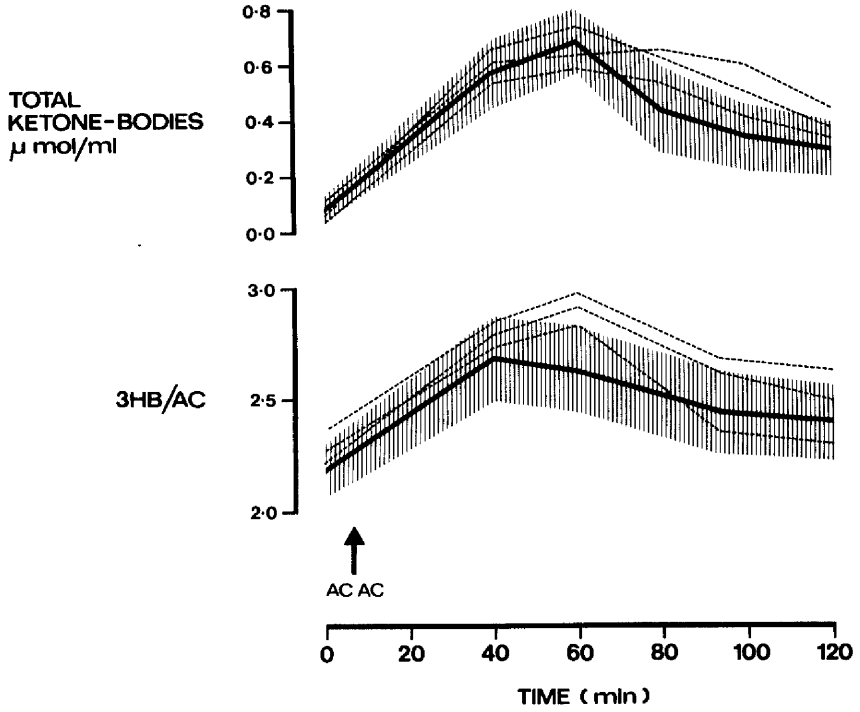
8.4 RESULTS OF ACETOACETATE TOLERANCE TESTS

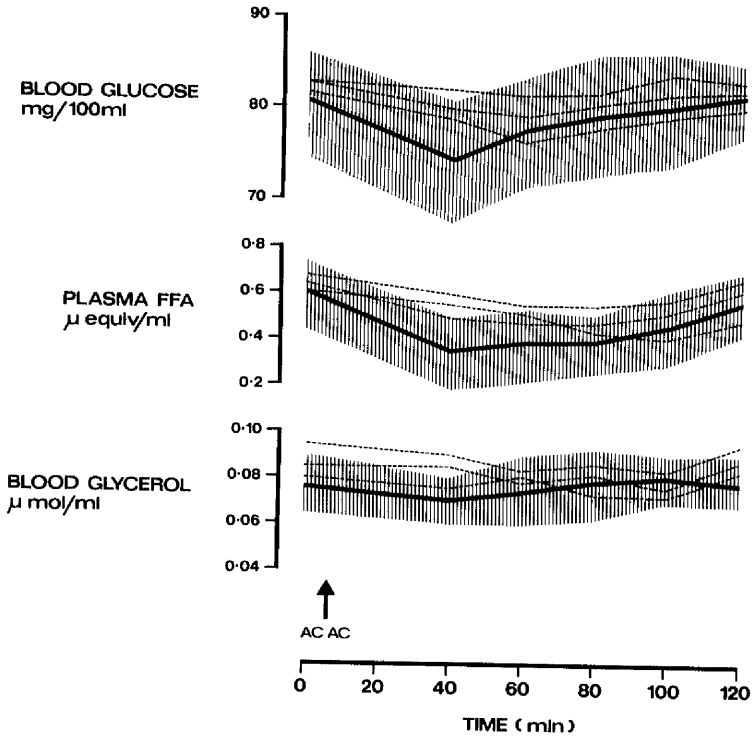
(a) Blood glucose: (Fig. 8:iii) Resting levels were similar in both groups. Blood-glucose fell in both patients and controls after taking acetoacetate but the fall was slightly greater in the controls so that the patients' glucose values were only significantly different from the control at 40 min. In both groups the concentration of glucose rose during the next 60 min and the values for patients fell within the range of control values.

(b) Blood glycerol and plasma FFA: (Fig. 8:iii) Resting levels of blood-glycerol and plasma FFA were similar in both groups. Ingestion of acetoacetate caused depression of lipolysis and of the blood concentration of glycerol and FFA fell in both groups. However, the fall was smaller in the patients so that the values of glycerol and FFA were significantly greater than the control mean at 40 min. The glycerol and FFA concentrations in the patients remained depressed at 60 min but since the control values had risen all the values were within the same range. Values of glycerol and FFA were similar through the remainder of the investigation.

Fig. 8:iii Changes in total ketone-bodies (μmol/ml) and 3-hydroxybutyrate:acetoacetate ratio before and after ingestion of 200 ml 0.4 M acetoacetate by 7 normal control subjects (means, solid line; hatching represents ± 2 SEM) and 3 hypopituitary patients (broken line).

Fig. 8:iv Changes in blood glucose (mg/100 ml), plasma FFA (μequiv/ml) and blood glycerol (μmol/ml) before and after ingestion of 200 ml 0.4M acetoacetate by 7 normal control subjects (means, solid line; hatching represents ± 2 SEM) and 3 hypopituitary patients (broken line).


TOTAL
KETONE-BODIES
μ mol/ml
0·8
0·6
0·4
0·2
0·0
3HB/AC
3·0
2·5
2·0
AC AC
0
20
40
60
80
100
120
TIME (min)


BLOOD GLUCOSE
mg/100ml
90
80
70
PLASMA FFA
μ equiv/ml
0·8
0·6
0·4
0·2
BLOOD GLYCEROL
μ mol/ml
0·10
0·08
0·06
0·04
AC AC
0
20
40
60
80
100
120
TIME (min)

(c) Ketone-bodies (acetoacetate and 3-hydroxybutyrate):

(Fig. 8:iv) Ketone-body levels were similar before ingestion of acetoacetate in the patients and controls. Total ketone-body concentration rose in both groups after taking acetoacetate so that there was little difference in their values at 40 and 60 min. However, the concentration of ketone-bodies fell to normal levels between 60 and 120 min in the controls but appeared to remain slightly elevated in the patients at 80 and 100 min.

The ratios of 3-hydroxybutyrate to acetoacetate were slightly higher in the patients throughout the investigation but the differences were most marked after 60 min.

8.5 DISCUSSION

The significance of these results is difficult to gauge because of the small number of patients tested. However, the figures show means ± 2SEM for the controls. Thus individual values for the patients are likely to be significant when they fall outside these limits. The results of the glucose tolerance tests in three hypopituitary patients agree with previous findings of a flattening of the glucose tolerance curve in hypopituitarism compared to controls (Stamp, 1965). The disappearance of glucose from the circulation may be linked with a greater utilisation of glucose as suggested by the elevated levels of lactate and pyruvate.

The response of the fat metabolites to oral glucose was similar to that previously reported (Stamp, 1965; Wilkinson, Hall, Cooper & Newell, 1970). The slightly greater fall in glycerol and FFA in the patients and the later return to normal levels supports previous suggestions of lability and impairment of normal fat mobilisation in patients with hypopituitarism.

However, the concentration of blood ketone-bodies in the patients was near to that of the controls throughout the investigation. This suggests that the extend of oxidation of ketone-bodies through the activation by glucose of the Krebs cycle in liver and other tissues is of a similar order.

The results might be interpreted as showing impaired fat mobilisation but nevertheless apparently normal ketone-body oxidation after a glucose load. This is in direct conflict with the apparently greater levels of circulating FFA and ketones during and after exercise by hypopituitary patients.

It is nevertheless possible to reconcile the facts using a possible explanation arising from the results of the acetoacetate tolerance tests, although the results are not conclusive, being gained from three patients only.

The slightly smaller fall in blood glucose in the hypopituitary patients suggests that they were less sensitive to ketone-bodies as a hypoglycaemic agent. Ketone-bodies normally depress blood-glucose by increasing insulin secretion (Madison et al., 1964) and it is possible that the hypopituitary patients secrete less insulin in response to exogenous ketones, thus depressing blood sugar less.

Ketone-bodies also normally depress FFA release (Jenkins, 1967) but the fall in glycerol and FFA was greater after ingestion of acetoacetate in the control subjects than in the patients, suggesting that the effect of ketone-bodies on lipolysis is less in hypopituitarism. The normal mechanism

is unlikely to be mediated via growth hormone (which the patients lack) since oral acetoacetate causes no change in HGH levels in man (Johnson et al., 1972).

Thus the greater levels of glycerol and FFA previously observed during exercise in patients with hypopituitarism could be explained if adipose tissue was less sensitive to inhibition of lipolysis by ketone-bodies and if there were a smaller degree of insulin secretion in response to post-exercise ketosis.

Despite the apparently normal depression of blood ketone-bodies in the patients during the OGTT there was an apparent impairment of ketone-body oxidation after ingestion of acetoacetate. In particular the ratio of 3-hydroxybutyrate: acetoacetate was greater in the patients, suggesting a lower removal rate at elevated levels of acetoacetate than when oxidation in the Krebs cycle is enhanced by glucose.

It may be that patients suffering from hypopituitarism have poor control over fat metabolism if and when lipolysis occurs in exercise, even though at rest the process is impaired. Thus the events taking place during exercise may be described in the following sequence:

(a) lipolysis caused during exercise by an unknown agent but possibly catecholamines, despite known decreased sensitivity.

(b) ketone-bodies produced by fatty acid oxidation fail to increase insulin secretion and fail to block lipolysis directly, therefore fatty acid levels continue to rise.

(c) after exercise fatty acid oxidation through the Krebs cycle in liver is impaired and ketone-body production continues.

8.6 SUMMARY

1. Three patients and nine controls were studied during oral glucose and acetoacetate tolerance tests. Venous blood samples were taken for estimation of growth hormone and insulin, pyruvate, lactate, glucose, FFA, glycerol and ketone-bodies.

2. The patients showed flattened glucose tolerance curves and a more lasting depression of FFA indicating greater insulin secretion or a more sensitive response to glucose than the controls.

3. The attenuated response of glucose and plasma FFA to acetoacetate in the patients suggests, however, that insulin was secreted to a smaller extent in response to exogenous ketones. The slightly greater blood ketone-bodies and the raised 3-hydroxybutyrate-acetoacetate ratio indicate poor oxidative removal of raised levels of acetoacetate in the patients.

CHAPTER 9

GROWTH HORMONE AND EXERCISE - II

The effect of moderate exercise on blood metabolites in patients with acromegaly.

9.1 INTRODUCTION

In previous studies on the metabolic role of HGH in exercise, described in Chapter 7, it was observed that patients with hypopituitarism, who are unable to increase their plasma levels of HGH nevertheless had a rise in the levels of FFA and ketone-bodies during and after exercise (Johnson, Rennie, Walton, & Webster, 1971). These results indicated that the development of post-exercise ketosis does not depend upon the presence of HGH; indeed the patients with hypopituitarism developed a greater degree of post-exercise ketosis than the controls. The difference could have been ascribed to differences in fitness but Winkler, Steele and Altzuler (1969) have suggested that HGH might cause increased re-esterification of FFA. The difference between the hypopituitary patients and the controls could have resulted from this effect. An opportunity to examine the possibility of such a metabolic effect of HGH during exercise is provided by the study of patients with acromegaly who secrete an excess of HGH.

9.2 SUBJECTS AND METHODS

9.2.1 The effect of 30 min of moderate exercise

Seven female patients (aged 24-57 yr) with acromegaly and nine controls (4 male and 5 female, aged 25-63 yr) were studied during and after a period of 30 min exercise. The patients had mean heights of 166 (± 2.4 cm SEM) and mean weights of 68.8 kg (± 6.2 kg SEM). The controls had a mean height of 167 cm (± 3.1 cm SEM) and weighed 70.2 kg (± 6.8 kg SEM). The patients had clinical symptoms of a pituitary tumour and acromegaly. Histological studies after operation indicated a pituitary tumour

of either eosinophilic, chromophobic or mixed type. All patients were studied before operation. Two patients only were receiving drugs (0.1 mg thyroxine daily) at the time of investigation. The control subjects were matched as far as possible for height and weight and none were known to have a metabolic disorder. Five were in hospital for treatment of conditions unrelated to acromegaly, including late onset epilepsy, cervical spondylosis and meningioma. No subject had undergone operation prior to study and they were not receiving drugs at the time of investigation. All subjects were eating normal diets with no restrictions.

9.2.2 The effect of a second period of moderate exercise

Two of the patients, both women aged 24 and 57, were also studied on a separate occasion when they rested for 90 min after exercise and then repeated the exercise test. Three controls (2 male and 1 female) (aged 25-37) were also studied.

9.2.3 The effect of surgical operation for removal of a pituitary tumour on growth hormone levels and the response to exercise.

Four patients (women aged 24-57) were investigated before operation and afterwards at periods between 3 months and 2 yr. On each occasion they exercised at the same rate. One patient, A.T. (57 yr) was investigated over a period of three years, once before operation and on three occasions afterwards.

9.2.4 The effect of successful treatment on the pattern of fat metabolism in acromegalic patients.

Three men (aged 36, 38 and 46) and one woman (aged 24, height 169 cm, weight 69 kg) who had been treated for acromegaly were studied. The men were 171, 168 and 105 cm tall and weighed 76, 69 and 77 kg respectively. They had undergone operations 2-3 yr previously which had been successful both clinically and judged from a marked decrease in HGH secretion. In two of these (one man and one woman) plasma levels of IRI were also measured.

9.3 PROCEDURE

All patients and controls were brought to the laboratory between 09.30 and 11.00 hr after overnight fasting. The investigation had been explained to all subjects and their consent obtained. The subjects were exercised for a period of 30 min on a bicycle ergometer (Elema Schönander constant load ergometer (EM 369) for most investigations) fixed at a work load of 500 ± 100 kpm/min.

Heart rate was recorded using an electrocardiograph (lead system II) during the investigations. A catheter was placed in the antecubital vein and blood samples were taken before exercise, at 5 min intervals during exercise and then at 30, 60 and 90 min afterwards. Samples were also taken at 120 and 150 min in the investigations upon three patients.

The pattern of sample timing was repeated in the investigation of the patients and controls during and after two periods of exercise. Blood samples were treated as described in Appendix I and analysed for lactate and pyruvate, acetoacetate and 3-hydroxybutyrate, glucose,

glycerol and plasma free fatty acids (FFA). Plasma HGH was analysed by a radio-immunoelectrophoretic method in all subjects and plasma IRI levels were measured by a charcoal immunoassay in five patients and four controls. Significance of difference was tested using the Mann-Whitney u non-parametric test for small samples.

9.4 RESULTS

9.4.1 THE EFFECT OF 30 MIN EXERCISE

9.4.1.1 Heart rate: (Fig. 9:i) The resting heart rates were 89 ± 5 SEM beats/min in the patients and 83 ± 3 SEM beats/min in the controls. In both groups heart rate increased rapidly during the first 5 min of exercise (controls 77%, patients 78%) after which the rate was steady until the end of exercise when a rapid fall occurred to within 10-20% 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.

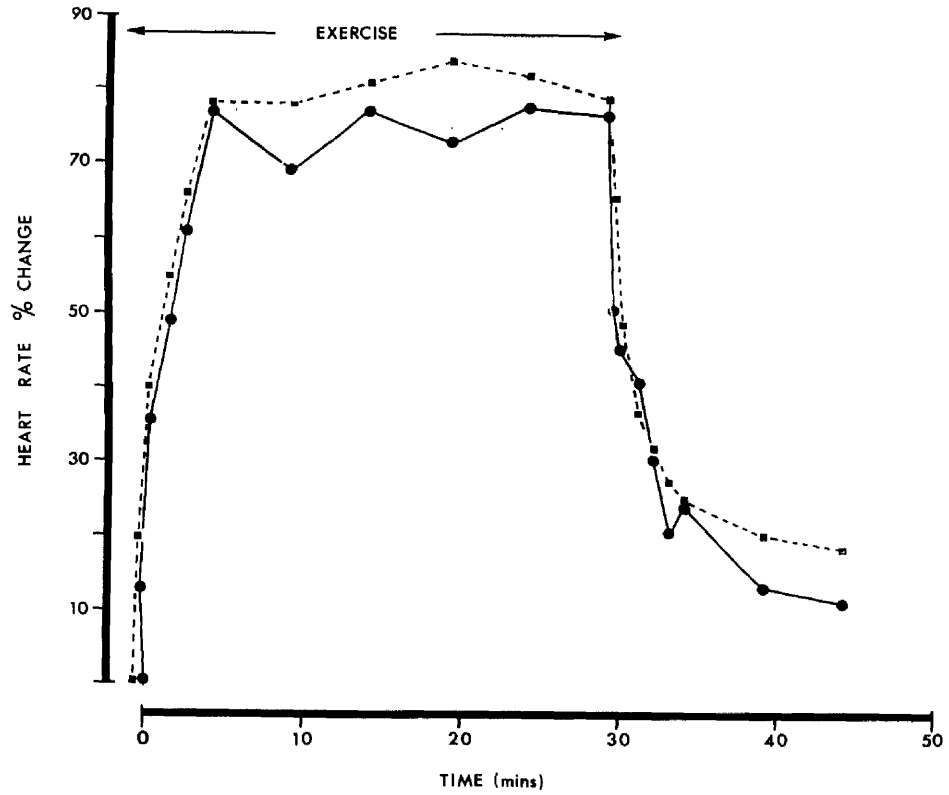
9.4.1.2 METABOLITES DURING AND AFTER 30 MIN EXERCISE

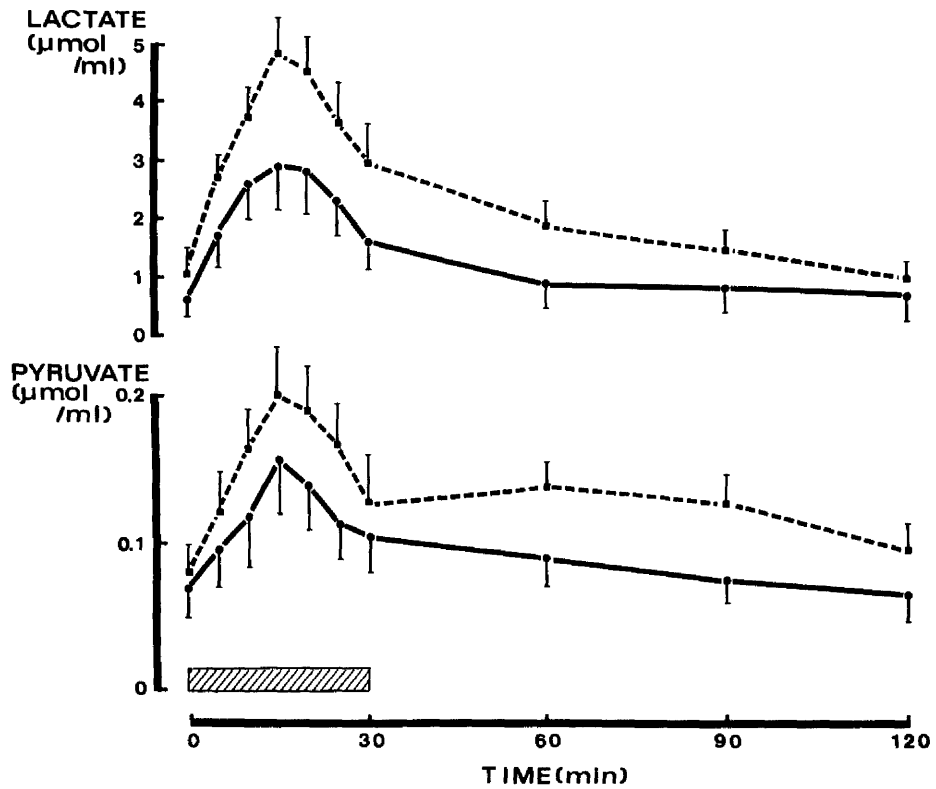
(a) Glucose: At rest the blood glucose concentration was 81 mg/100 ml (± 7 SEM) in the patients and 75 mg/100 ml (± 4 SEM) in the controls. At the end of exercise the values were 84 mg/100 ml (± 8 SEM) in the patients and 79 mg/100 ml (± 6 SEM) in the controls. There was little change in the values in either group during the remainder of the study.

(b) Pyruvate: (Fig. 9:ii) Resting blood pyruvate concentrations were similar in both groups. Exercise caused a

Fig. 9:i Mean heart rate % changes in 8 control subjects (⊙ —— ○) and 7 acromegalic patients (▩ ---- ▩) during and after 30 min exercise. There were no significant differences between the changes observed at any time.

Fig. 9:ii Blood lactate and pyruvate levels umol/ml (means ± 1 SEM) in 9 control subjects (⊙ —— ⊙) and 7 acromegalic patients (▩ ---- ▩) during and after 30 min exercise.


EXERCISE
HEART RATE % CHANGE
90
70
50
30
10
0
10
20
30
40
50
TIME (mins)


LACTATE
(µmol /ml)
5
4
3
2
1
0
PYRUVATE
(µmol /ml)
0.2
0.1
0
0
30
60
90
120
TIME(min)

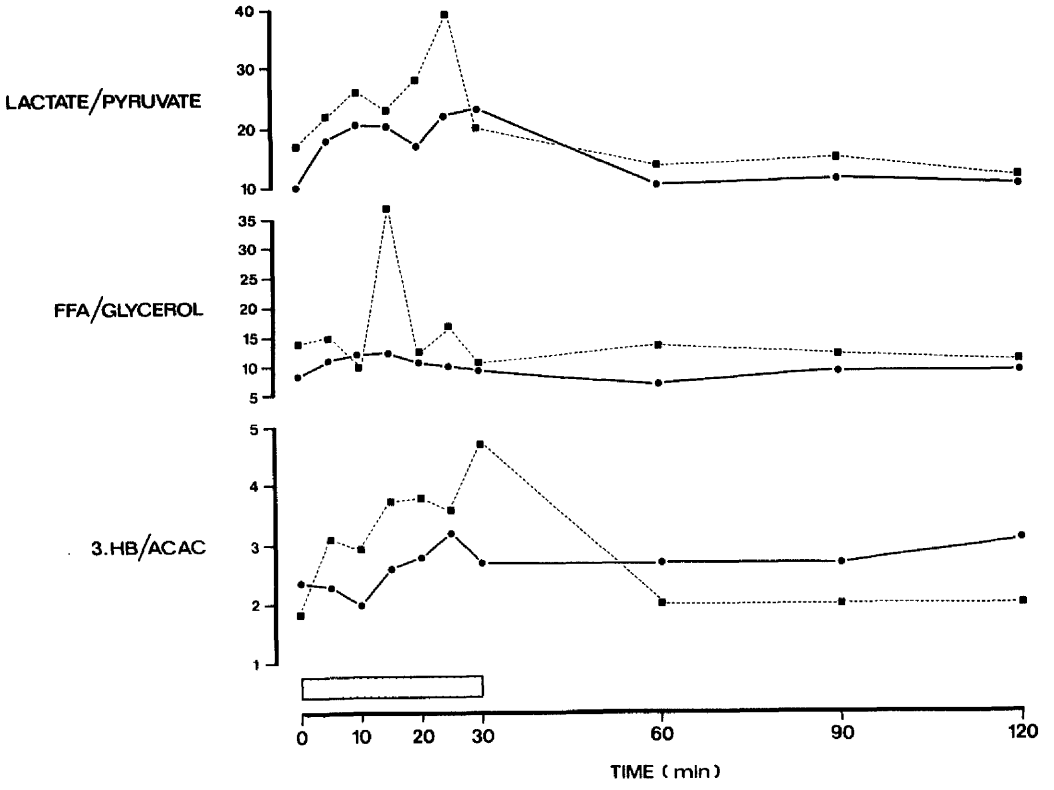
rapid rise in pyruvate values to a peak at 15 min which was slightly greater in the patients, but the difference was not significant ($P > 0.05$). Pyruvate concentrations fell during the second half of the exercise period in each group but in the acromegalics they were still significantly elevated above the resting level 60 min after the end of exercise. In the control subjects pyruvate levels had almost fallen to resting levels 30 min after exercise. The levels in the two groups were significantly different at the end of exercise and at 30 min ($P < 0.05$) and 60 min ($P < 0.05$) after exercise; after 90 min, however, there was no significant difference ($P > 0.05$).

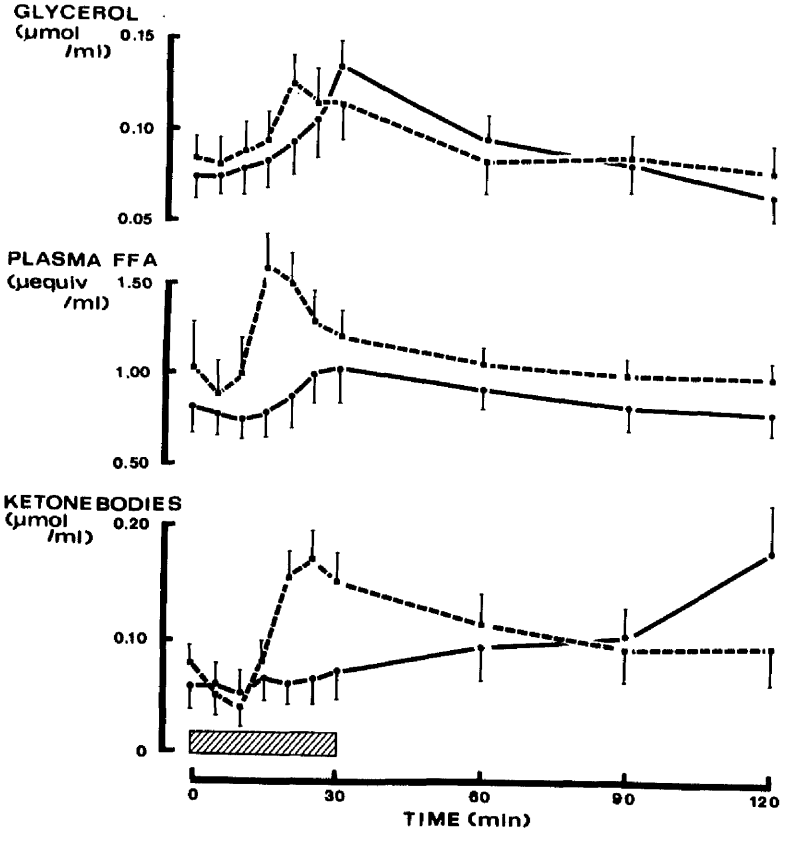
(c) Lactate: (Fig. 9:ii) Resting concentrations of lactate were similar in both groups. Rapid elevation of blood lactate values to a peak at 15 min of exercise occurred in both groups, the maximum being significantly greater in the acromegalics. The concentrations in the acromegalics remained significantly different ($P < 0.05$) from their resting levels for the succeeding 15 min of exercise and for 60 min after exercise. The controls, however, showed a more rapid return to approximately pre-exercise levels so that there was no significant difference ($P > 0.05$) between the resting value and the value 30 min after the end of exercise.

(d) Lactate-Pyruvate ratio: (Fig. 9:iii) The ratio was elevated in the patients compared to the controls at rest and during the first 25 min of exercise. In particular, the value of the ratio was almost twice the control value in the patients at 25 min of exercise. However, the ratio fell rapidly in the remainder of exercise and there was little difference in the

Fig. 9:iii Mean changes in ratios of lactate and pyruvate; FFA and glycerol; and 3-hydroxybutyrate and acetoacetate during and after 30 min exercise in 8 control subjects (● — ●) and 7 acromegalic patients (■ ---■).

Fig. 9:iv Blood glycerol (μmol/ml) plasma FFA (μequiv/ml) and blood ketone-bodies (μmol/ml) (means ± 1 SEM) in 9 control subjects (● — ●) and 7 acromegalic patients (■ ---■) during and after 30 min exercise.


LACTATE/PYRUVATE
40
30
20
10
FFA/GLYCEROL
35
30
25
20
15
10
5
3.HB/ACAC
5
4
3
2
1
0
10
20
30
60
90
120
TIME (min)


GLYCEROL
(μmol /ml)
0.15
0.10
0.05
PLASMA FFA
(μequiv /ml)
1.50
1.00
0.50
KETONE BODIES
(μmol /ml)
0.20
0.10
0
0
30
60
90
120
TIME (min)

post-exercise period between the values of the ratio in the control group and in the patients.

(e) Glycerol: (Fig. 9:iv) Resting glycerol concentrations were not significantly different between the groups. Blood glycerol increased during exercise earlier in the patients. The peak levels were not significantly different but since they occurred at different times, there was a significant difference in the 20 and 30 min levels of the two groups (20 min, $P < 0.01$; 30 min, $P < 0.05$).

(f) FFA: (Fig. 9:iv) Resting plasma FFA concentrations were not significantly different between the two groups. The values fell slightly in the early part of exercise and rose as the exercise continued. The peak which was most marked in the patients occurred at 15 min and fell during the remainder of the exercise. The control group showed a much smaller peak at the end of exercise and the levels returned to pre-exercise levels 90 min after the end of exercise. The differences in levels of FFA between the two groups were significant at 15, 20 and 25 min of exercise ($P < 0.05$) and their maximum values highly significantly different ($P < 0.01$).

(g) FFA-Glycerol ratio: (Fig. 9:iii) The ratio was significantly elevated at rest in the patients compared with the controls. During exercise the ratio in the patients rose in an erratic fashion so that it was three times the value of the control subjects at 15 min of exercise. In both groups the ratio fell in the last 15 min of exercise but the value in the patients remained slightly elevated throughout the post-exercise period.

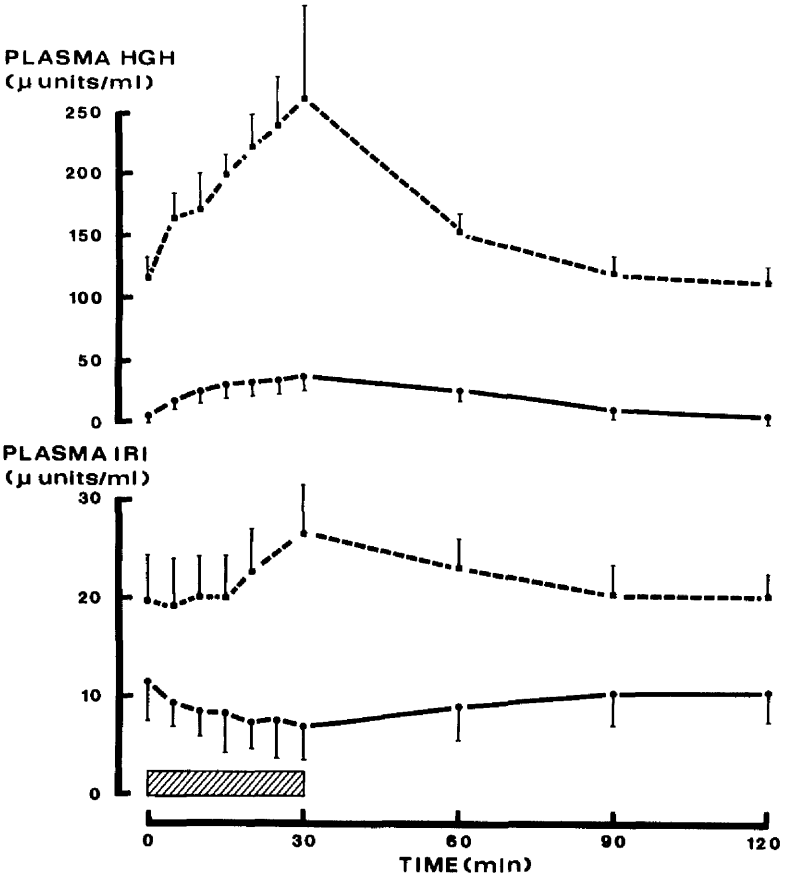
(h) Ketone-bodies: (Fig. 9:iv) Resting concentrations of ketone-bodies (acetoacetate plus 3-hydroxybutyrate) were similar in both groups but changes during exercise were very different. After a fall in the first 10 min the values in the patients rose rapidly to a peak 25 min after the beginning of exercise and then fell in the last 5 min of exercise. In the control group ketone-body concentrations changed little during the period of exercise but in the 90 min following exercise rose to nearly 300% of the control resting value. There was no difference in the response of the male and female controls. The concentrations of ketone-bodies in the two groups were significantly different at 15, 20, 25, 30 and 120 min ($P < 0.05$).

(i) 3-Hydroxybutyrate-Acetoacetate ratio: (Fig. 9:iii) The ratio was slightly lower in the acromegalic patients at rest. During exercise, however, it rose rapidly to a maximum value at 30 min. The control value fell at first but then also rose, less rapidly than in the patients, to a maximum at 25 min. In both groups the ratios fell after the maximum but the difference at 30 min was highly significant. In the post-exercise period the ratio was slightly higher for the control subjects, since the value fell more rapidly in the patients.

9.4.1.3 HORMONES DURING AND AFTER 30 MIN EXERCISE

(a) Plasma HGH: (Fig. 9:v) The acromegalic patients showed characteristically high resting levels of HGH (range 32-317 μ units/ml) while the control group had normal resting levels (5-10 μ units/ml). In both groups exercise caused rises in HGH levels to a maximum at 30 min of exercise. Although the changes were proportionately smaller in the acromegalics the changes in absolute values were considerable

Fig. 9:v Plasma HGH (μunits/ml, means ± 1 SEM) in 9 control subjects (● — ○) and 7 acromegalic patients (■ --- □) and plasma IRI (μunits/ml, means ± 1 SEM) in 5 control subjects (○ — ○) and 4 acromegalic patients (■ --- □) during and after 30 min exercise.


PLASMA HGH
(μ units/ml)
250
200
150
100
50
0
PLASMA IRI
(μ units/ml)
30
20
10
0
0
30
60
90
120
TIME (min)

(up to 200 μ units/ml increase). In patients and controls HGH levels fell in the period following exercise to approximately pre-exercise levels at 90 min after the end of exercise.

(b) Plasma IRI: (Fig. 9:v) Resting concentrations of IRI were significantly higher in the acromegalic patients than in the two controls ($P < 0.05$). The concentrations fell during exercise in the control subjects but rose in the acromegalic patients so that the difference between the two groups at the end of exercise was considerable, the levels in the acromegalic patients being more than double those in the controls. In both groups plasma insulin concentration returned to resting values by 60 min after exercise.

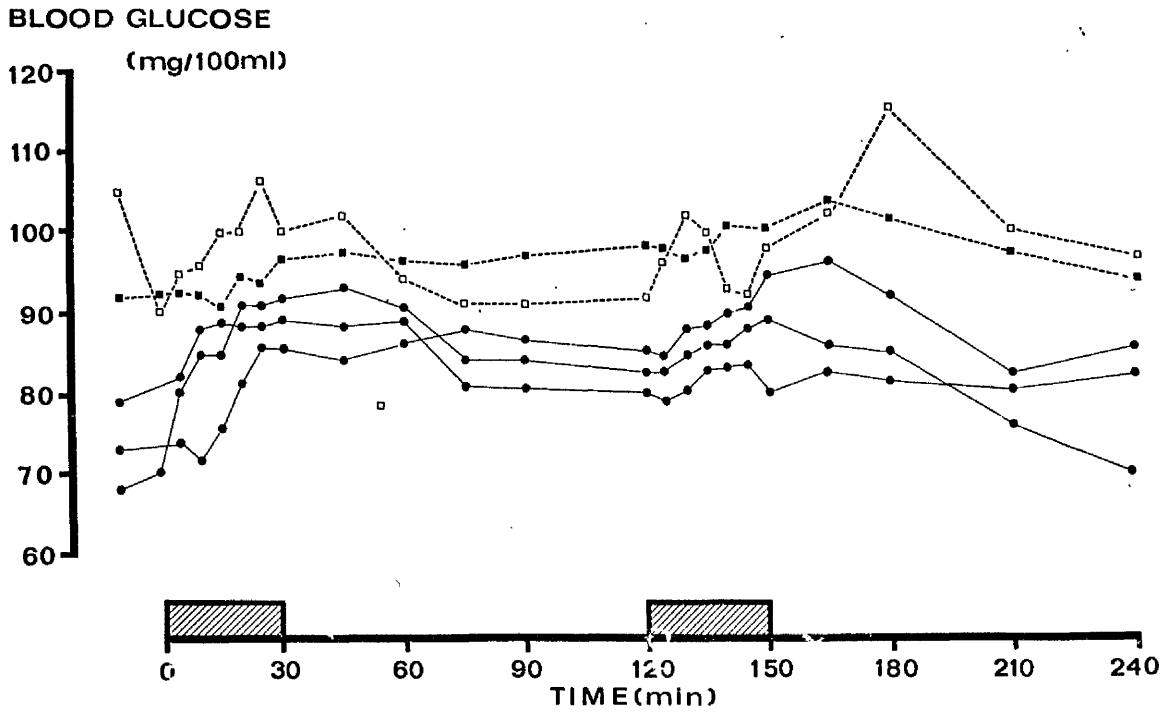
9.4.2 THE EFFECT OF A SECOND PERIOD OF EXERCISE

A second period of exercise caused changes in metabolites and hormones of a similar pattern to those occurring with one period. Blood glucose (Fig. 9:vi) concentration was higher throughout the investigation in the acromegalics. In both groups there was a slight rise in glucose during exercise. Although changes in lactate and pyruvate (Fig. 9:vii) were less in the second period, changes in blood ketone-bodies (Fig. 9:viii) and I RI (Fig. 9:ix) were much greater. Plasma HGH levels responded in a similar way during the second period as they had in the first but the rise was greater.

Ketone-body concentrations rose in the control subjects in the 90 min from the end of the first period of exercise and fell progressively during the second period. There was a considerable degree of post-exercise ketosis. The patients showed a decrease in

Fig. 9:vi Blood glucose changes mg/100 ml (mean ± 1 SEM) in 3 control subjects (o — o) and 2 acromegalic patients (□ --- □, ▨ --- ▨) during and after two 30 min periods of exercise.

Fig. 9:vii Blood lactate changes μmol/ml (mean ± 1 SEM) in 3 control subjects (o --- o) and 2 acromegalic patients (□ --- □, ▨ --- ▨) during and after two 30 min periods of exercise.


BLOOD GLUCOSE
(mg/100ml)
120
110
100
90
80
70
60
0
30
60
90
120
150
180
210
240
TIME(min)

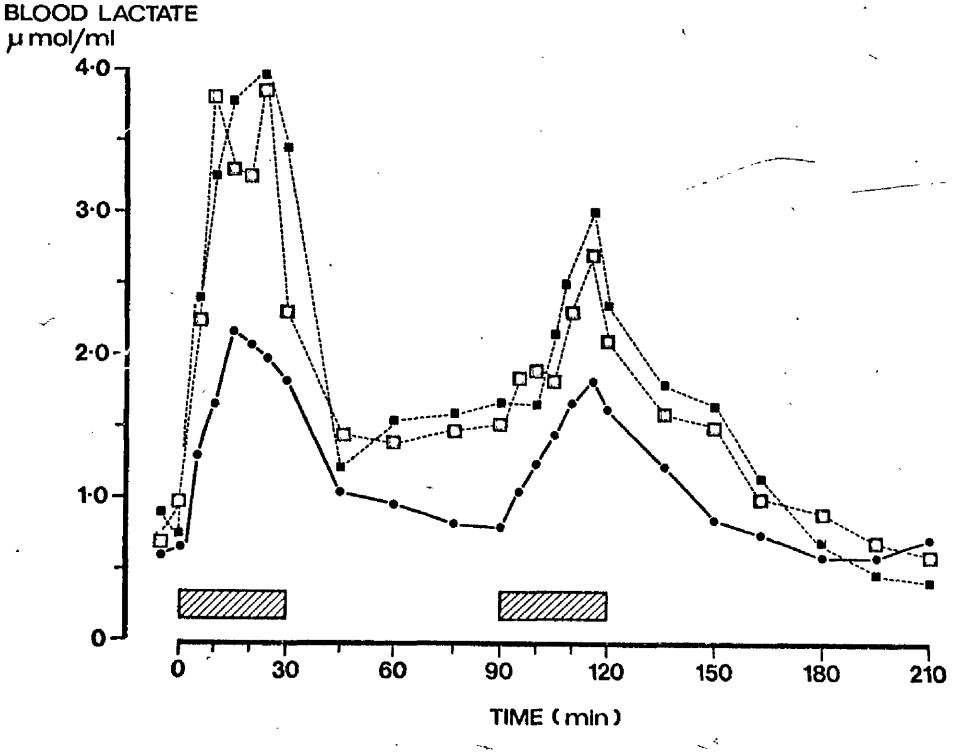
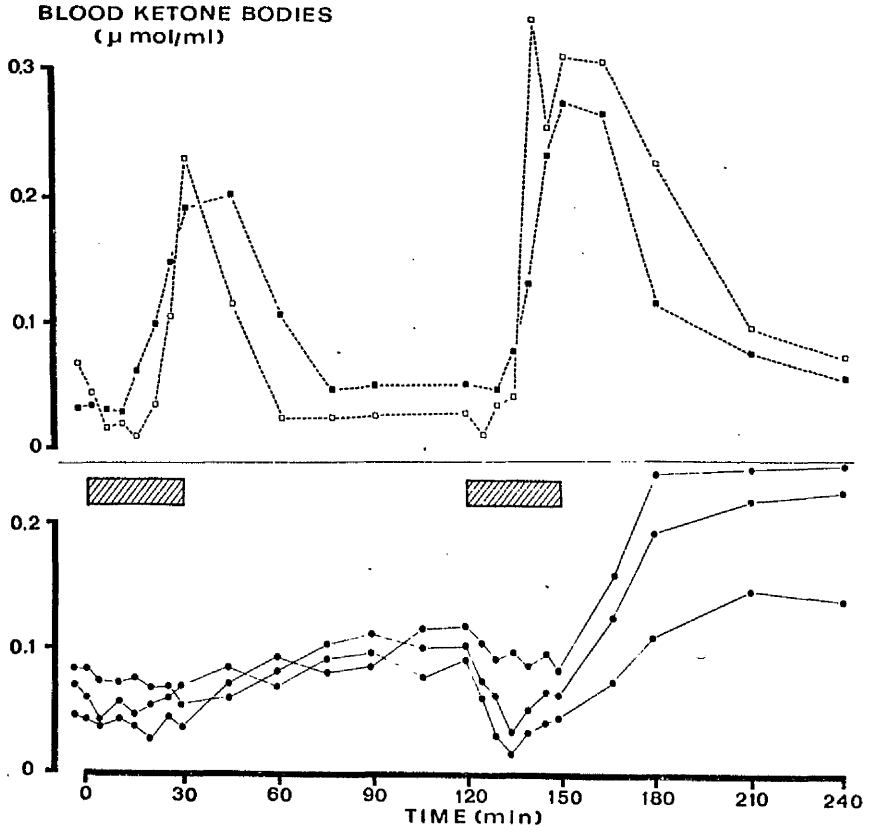
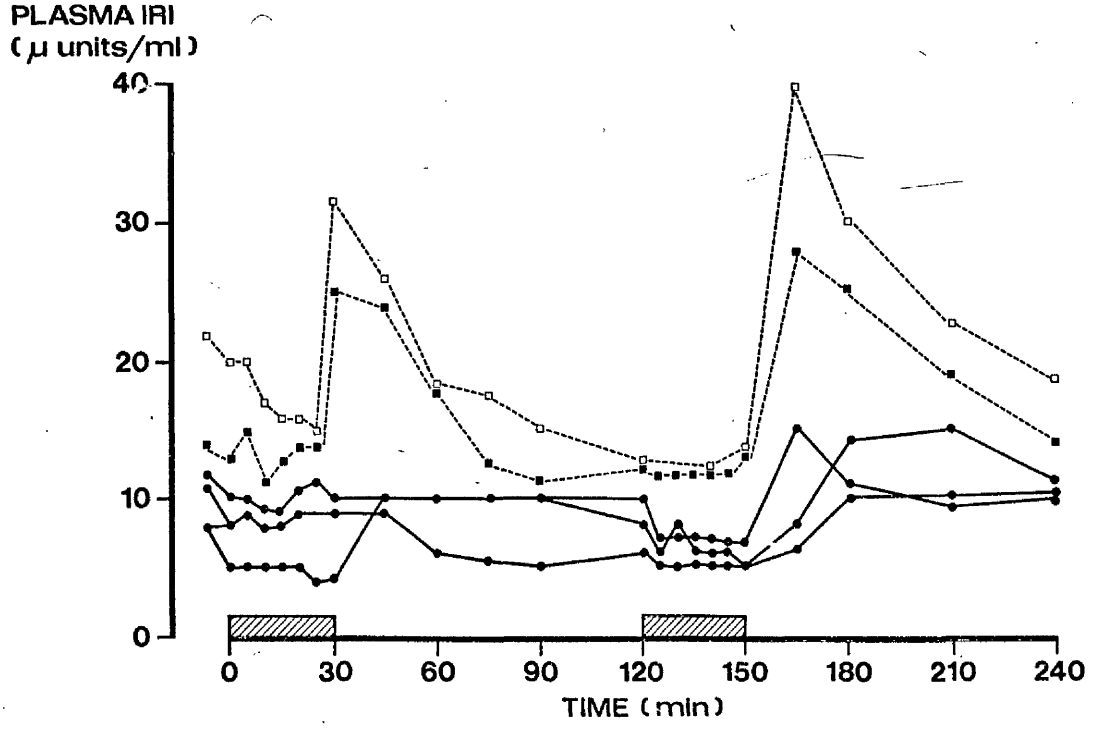

BLOOD LACTATE
μmol/ml
4·0
3·0
2·0
1·0
0
0
30
60
90
120
150
180
210
TIME (min)

Fig. 9:viii Blood ketone-body changes umol/ml (mean ± 1 SEM) in 3 control subjects (o — o) and 2 acromegalic patients (□ --- □, ▨ --- ▨) during and after two 30 min periods of exercise.

Fig. 9:ix Plasma IRI changes uunits/ml (mean ± 1 SEM) in 3 control subjects (o — o) and 2 acromegalic patients (□ --- □, ▨ --- ▨) during and after two 30 min periods 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)


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

levels of ketone-bodies after the first period of exercise but nevertheless the concentrations rose rapidly after 15 min of the second period. The values remained elevated for 15 min following the second period and fell rapidly in the remaining 75 min of the investigation.

Human growth hormone levels rose to a greater extent in both groups during a second period of exercise compared with the first. They nevertheless fell rapidly after the second exercise period.

The insulin concentrations fell in the control subjects during the second period of exercise and rose above their initial resting values in the post-exercise period. However, although the two patients showed little change in insulin concentration during the second period of exercise, there was a large rapid rise in their insulin values within 15 min of the end of the second period. The concentrations of IRI fell steadily in the succeeding 75 min.

9.4.3 THE EFFECT OF SURGICAL OPERATION ON HGH AT REST AND DURING EXERCISE (TABLE 9:i)

(a) Plasma HGH: In four patients who had undergone either cryostatic ablation or surgical removal of their tumour, the resting levels of growth hormone fell compared with the pre-operation values.

In one patient, J.B. (aged 24) the levels fell dramatically both at rest and during exercise. In addition the HGH response to glucose became normal and normal menstruation recurred (see later in chapter).

Table 9:i The effect of surgical ablation of pituitary tumour on HGH levels at rest and during exercise.

Plasma HGH (u units/ml)

Patient	Resting		Maximum during exercise	
	Pre-op	Post-op	Pre-op	Post-op
J.B.	68	8	94	27
J.G.	317	302	500	500
M.S.	132	68	178	102
A.T.	116	105	348	250
A.T. 1 yr post-op		47		130
2 yr post-op		31		68
Mean (excluding A.T. 1 & 2 yr)	158	120	280	219
SEM	±55	±64	±90	±104
Significance: paired Student's t test		$P < 0.05$		$P < 0.05$

Controls:

Mean	5.0	38
S.E.M.	±4.8	±12

The other patients showed a smaller decrease in levels of HGH at rest and during exercise but the change was significant on the basis of a paired t-test ($P < 0.05$).

(b) Metabolite changes: The metabolic changes during and after exercise in the acromegalic patients, in whom the HGH concentrations fell after operation but were still much outside the normal range, were of a similar pattern to the changes observed before operation. Thus there was massive fat mobilisation during exercise, a large increase in blood ketone-bodies at the end of exercise and little post-exercise ketosis.

The changes with exercise in patients who showed a fall in H GH levels to normal values after operation are discussed below.

9.4.4 THE EFFECT OF SUCCESSFUL TREATMENT ON THE PATTERN OF FAT METABOLISM IN ACROMEGALIC PATIENTS (Fig.9:x; 9:xi; Tables 9:ii;9:iii)

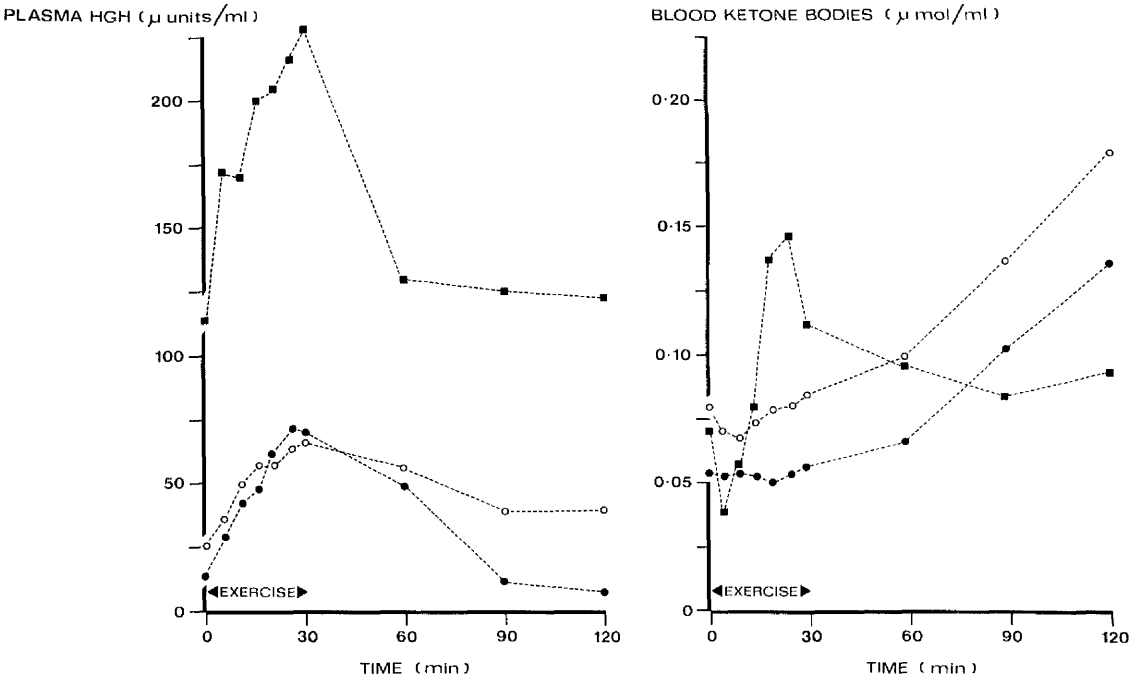
The pattern of changes in HGH (4 patients), insulin (2 patients) and metabolites (4 patients) was very similar, after successful treatment of acromegaly, to the changes observed in normal controls. Resting levels of HGH were within the normal range although they rose to a greater extent during exercise. The extensive lipolysis observed in the untreated patients during exercise was much attenuated in the treated patients and the rise in ketone-bodies within the exercise p eriod was abolished. However, post-exercise ketosis was observed to some extent in all four patients.

The insulin response to exercise in the two treated patients examined was of the normal pattern with a fall during exercise and a post-exercise elevation.

The changes were particularly striking in the case of J.B. who

Fig. 9:x Plasma HGH (μunits/ml) and blood ketone-bodies (μmol/ml) during and after 30 min exercise. Mean values for 7 acromegalic patients (▣) and individual values for 2 patients (●, ○) who had been successfully treated for acromegaly.

Fig. 9:xi Plasma immunoreactive insulin (μurits/ml) and blood ketone-bodies (μmol/ml) during and after 30 min exercise. Individual values in two acromegalic patients (▣, □) two control subjects (●, ○) and one acromegalic patient who had been treated successfully (△).


PLASMA HGH (μ units/ml)
200
150
100
50
0
◀EXERCISE▶
0
30
60
90
120
TIME (min)
BLOOD KETONE BODIES (μ mol/ml)
0·20
0·15
0·10
0·05
0
◀EXERCISE▶
0
30
60
90
120
TIME (min)

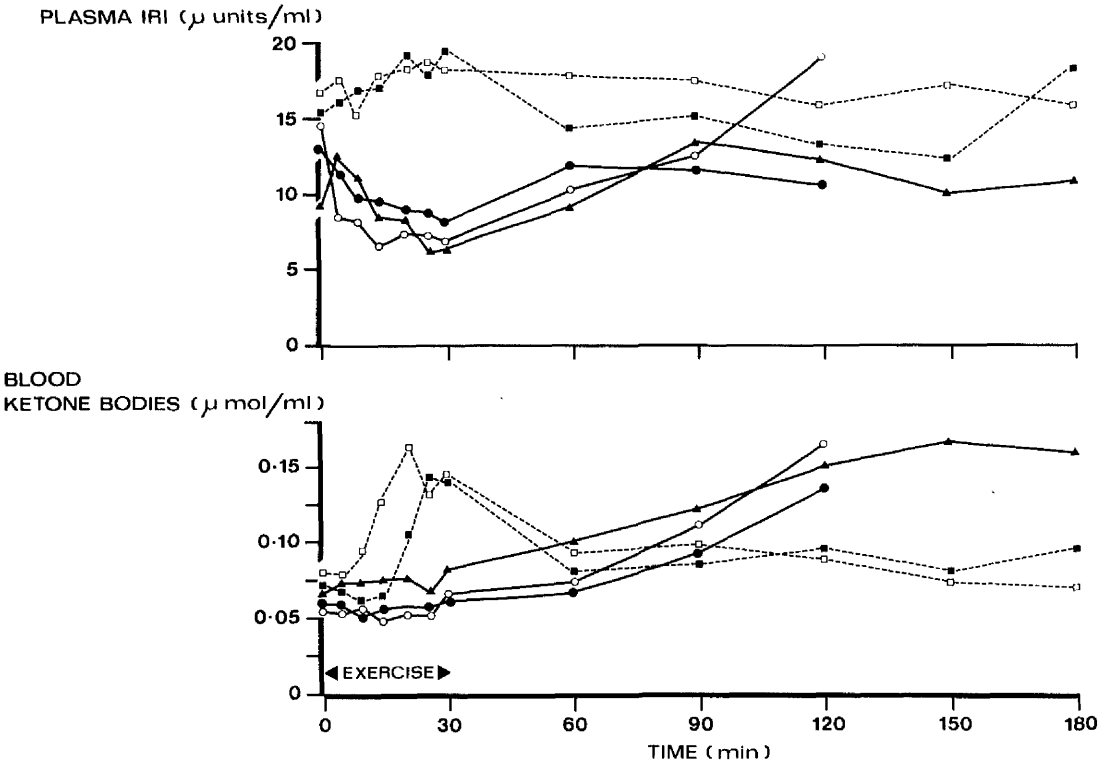

PLASMA IRI (μ units/ml)
20
15
10
5
0
BLOOD
KETONE BODIES (μ mol/ml)
0·15
0·10
0·05
0
◀EXERCISE▶
0
30
60
90
120
150
180
TIME (min)

TABLE 9:ii. Changes in HGH, IRI, plasma FFA and blood ketone-bodies during and after exercise by 7 patients suffering from acromegaly (untreated) and 4 others successfully treated (treated).

	Rest		15 min Exercise		30 min Exercise		30 min Post-Exercise		90 min Post-Exercise	
	Untreated (7)	Treated (4)	Untreated (7)	Treated (4)	Untreated (7)	Treated (4)	Untreated (7)	Treated (4)	Untreated (7)	Treated (4)
Plasma HGH (u units/ml) Means ± SEM	112 ±14	18 ±8	195 ±24	47 ±9	242 ±97	78 ±5	136 ±12	53 ±6	118 ±15	22 ±10
Plasma IRI (u units/ml) Means ± SEM	19 ±4	12 ±6	19 ±4	10 ±3	24 ±8	8.5 ±2	21 ±5	13 ±4	20 ±4	12 ±5
Plasma FFA (u equiv/ml) Means ± SEM	1.05 ±0.25	0.892 ±0.097	1.55 ±0.19	0.943 ±0.056	1.28 ±0.17	1.02 ±0.082	1.16 ±0.12	0.960 ±0.101	1.08 ±0.14	0.910 ±0.010
Blood ketone-bodies (u mol/ml) Means ± SEM	0.082 ±0.023	0.074 ±0.018	0.093 ±0.036	0.069 ±0.021	0.151 ±0.034	0.082 ±0.028	0.113 ±0.042	0.108 ±0.027	0.094 ±0.038	0.148 ±0.051

Table 9.iii

Changes in HGH, IRI, FFA and blood ketone-bodies in one patient (J.B.) during and after exercise before and after treatment for acromegaly by cryostatic ablation of a pituitary tumour.

	Rest		15 min Exercise		30 min Exercise		30 min Post-Exercise		90 min Post-Exercise	
	Before	After	Before	After	Before	After	Before	After	Before	After
Plasma HGH (μ units/ml)	68	8	80	17	94	27	81	12	72	7
Plasma IRI (μ units/ml)	14	10	12	9	16	7	18	12	17	11
Plasma FFA (μ equiv/ml)	0.97	0.78	1.24	0.99	1.042	1.11	0.92	0.86	0.88	0.811
Blood Ketone-bodies (μ mol/ml)	0.085	0.081	0.100	0.072	0.131	0.088	0.098	0.121	0.092	0.150

had exercised both before and after her operation, which had resulted in a very marked clinical improvement (Table 9:iii).

9.5 DISCUSSION

The diagnosis of acromegaly was confirmed in the patients by the abnormally high levels of HGH at rest. Exercise caused a marked rise in HGH in the patients with acromegaly, suggesting that their HGH secretory centres were able to respond to the changes associated with exercise. This effect could be mediated by an increase in cortisol (Greenwood & Landon, 1966), by an increase in peripherally secreted catecholamines acting at receptor centres (Blackard & Hubbell, 1970), or through the central action of neurotransmitters alone (Sherman & Kolodny, 1971).

The percentage changes in heart rate with exercise in both groups of subjects was similar, suggesting that the work done was the same.

During the first 10 min of exercise levels of plasma FFA and ketone-bodies fell in both groups. This has been ascribed to an inhibitory effect of lactate on mobilisation of fat from adipose tissue and also to a direct anticatabolic effect of lactate on the liver (Issekutz & Miller, 1962; Houghton, Hawkins, Williamson & Krebs, 1971). However, in a previous study of exercise in patients with hypopituitarism, no depression of FFA occurred despite the elevation of lactate to levels above those of controls (Johnson et al., 1971). The initial changes in levels of FFA and ketone-bodies have also been ascribed to increased peripheral utilisation (Drury, Wick & Mackay, 1941; Havel, Naimark & Borchgrevink, 1963) and this could be related, in the acromegalics, to a greater peripheral muscle blood flow which has been demonstrated at rest

in such patients (Butterfield, Garratt & Whichelow, 1963).

After 10 min there were marked differences between the two groups in metabolite concentration in the blood. The acromegalic patients had a substantially greater degree of fat mobilisation than the controls, both in terms of absolute levels of glycerol and FFA and of the raised FFA-glycerol ratio.

The increased lipolysis resulted in the blood ketone-body levels reaching a peak during exercise, a phenomenon which has not been observed in any other subjects. The greater increase in fat metabolism in the acromegalic patients might be explained by their high levels of HGH, a known lipolytic agent, but its absence does not prevent lipid mobilisation during exercise (Troyer, Friedberg, Horton & Bogdonoff, 1966; Johnson et al., 1971). It may, nevertheless, be important in facilitating the mobilisation of depot fat (Hunter et al., 1965). This view is strengthened by the present observations that similar patterns of lipolysis occurred in post-operative patients, in whom HGH levels were still elevated and that the changes were exaggerated by a second period of exercise but that patients whose HGH levels were normal following operation showed a normal lipolytic and metabolic response to exercise. It is possible that growth hormone sensitises adipose tissue to the action of other adipokinetic hormones since hypophysectomy is followed by a decreased sensitivity (Goodman & Knobil, 1959).

After 20-25 min of exercise the high levels of glycerol, plasma FFA and ketone-bodies in the acromegalics were depressed. There was also no rise in ketone-bodies after exercise as in the

control group (Johnson et al., 1969; Courtice & Douglas, 1936; see also Chapters 1, 2, 3 & 4). One explanation for this depression might be the suggestion that HGH has a dual action on fat metabolism (Winkler, Steele & Altszuler, 1968; Johnson et al., 1971). Initially high levels of HGH might exaggerate fat mobilisation during exercise as observed in the present study and at a later stage it might cause inhibition by accelerating the re-esterification of FFA in adipose tissue. The dual action could be mediated by two polypeptide sub-units which may act antagonistically on fat and carbohydrate breakdown and synthesis (Bornstein, Krahl, Marshall, Gould & Armstrong, 1968).

An alternative explanation depends upon the marked differences we have observed in IRI, which fell in the controls as shown by other workers (Devlin, 1963; Cochran et al., 1966) and rose in the acromegalic patients. The increase in the acromegalics might be a response to the rapid increase in FFA and ketone-bodies which are known to cause insulin release (Madison et al., 1964). A major effect of insulin is to facilitate esterification of FFA to fat (Bieberdorf, Chernick & Scow, 1970) and it is, therefore, probable that the marked depression of glycerol, FFA and ketone-bodies in the acromegalics after exercise is related to their much higher IRI levels. The sudden decrease in the ratio of 3-hydroxybutyrate-acetoacetate also supports the decreased production of ketone-bodies from circulating FFA. It is also possible that the fall could be caused by a rapid incorporation of two-carbon fragments into fatty acids and phospholipids, caused by insulin, but this is less likely.

The changes in HGH and IRI and the concomittant disordered fat metabolism were exaggerated by a second period of exercise in two patients. The continuing high levels of HGH in some patients who had undergone operation were always accompanied by the pattern of massive exercise lipolysis and lack of post-exercise ketosis. The key factor in the cause of the observed changes in fat metabolites and insulin appears to be HGH since the characteristic "acromegalic" pattern was not observed in four treated patients in whom the HGH response to exercise was normal.

The patients also showed elevated levels of lactate and pyruvate. An increased rate of fatty acid oxidisation increases the formation of acetyl CoA and its subsequent oxidation in the Krebs cycle. This may cause a block to pyruvate oxidation and if insufficient oxalacetate is available, therefore, build-up of lactate and pyruvate. This seemed to be the case for the acromegalic patients in whom the lactate-pyruvate ratio rose to twice the resting value after the peak of lipolysis shown by FFA and glycerol concentrations and the maximum FFA-glycerol ratio.

The decline in lactate and pyruvate levels later in the exercise period has been previously reported in other subjects and may reflect increased blood flow in the exercising muscles (Harris, Bateman, Bayley, Donald, Gloster & Whitehead, 1968; Johnson et al., 1971).

A second period of exercise resulted in lower levels of lactate and pyruvate in both patients and normal controls. This was probably related to a decrease in muscle glycogen stores after the first period of exercise and this possibility

would explain the increased lipolysis in the second period.

Although the present observations might be consistent with the suggestions (see Chapter 7) that HGH has a delayed or post-exercise inhibitory effect on fat metabolism (Winkler, Steele & Altszuler, 1968; Johnson et al.,1971). The acromegalic subjects also showed a marked rise in insulin levels compared with controls during and after exercise. This observation suggests that insulin has a major effect upon fatty acid mobilisation and re-esterification during and after exercise by acromegalics. This action of insulin appeared independent of any change in blood glucose levels, which remained substantially unaltered, but might be triggered by high concentrations of HGH since it disappeared in treated acromegalic patients with normal levels of HGH.

9.6 SUMMARY

1. Seven patients with active acromegaly, four successfully treated patients and nine controls were studied before, during and after 30 min of moderate steady exercise on a bicycle ergometer. Venous blood samples were taken for estimation of growth hormone (HGH), pyruvate, lactate, glucose, free fatty acids (FFA), glycerol and ketone-bodies. Plasma immunoreactive insulin (IRI) was measured in four patients and five controls.

2. Exercise caused a rise in HGH levels in the acromegalic patients, despite their pituitary tumour, and also in the control subjects. The patients who had been successfully treated for acromegaly showed changes similar to those in the controls. Levels of IRI fell during exercise in the control subjects, but rose in the acromegalic patients.

3. Levels of glycerol, FFA and ketone-bodies rose rapidly to a maximum during exercise in the acromegalics and appeared to be suppressed before the end of exercise. There was no increase in the concentration of ketone-bodies after exercise. In the control subjects there was a gradual increase in glycerol and FFA levels towards the end of exercise but no change in ketone-bodies occurred until the post-exercise period, when ketone-body concentrations rose.

4. It is concluded that exercise causes remarkable differences in metabolite concentrations in the blood of acromegalic patients compared with controls with the concentration of fat metabolites reaching a maximum then decreasing during the period of constant exercise. There was also elevation instead of the normal fall, of plasma IRI activity and it is suggested that the decrease in the concentration of fat metabolites occurred because of the rise of insulin concentration. It is further suggested that in acromegaly insulin retains its effect on re-esterification of fat in spite of resistance to its effects on carbohydrate metabolism.

CHAPTER 10

CARBOHYDRATE AND FAT METABOLISM IN ACROMEGALIC PATIENTS AT REST: GLUCOSE AND ACETOACETATE TOLERANCE

10.1 INTRODUCTION

Glucose tolerance is decreased in acromegaly, probably because of the inhibition by growth hormone of insulin induced uptake of glucose by peripheral tissues (Ikkos, Luft, Gemzell & Almqvist, 1962). The blood levels of intermediates of carbohydrate metabolism appear to be normal in acromegalic subjects during glucose tolerance (Ikkos et al., 1962). Plasma growth hormone concentration is not depressed by oral glucose although there is an exaggerated increase in the insulin response (Karam, Grodsky & Forsham, 1963).

There is less information available on the effect of glucose on FFA levels in acromegaly. The insulin induced depression of FFA in normal subjects is said to be independent of HGH (Rabinowitz & Zieler, 1962) suggesting that the insulin mediated response should occur normally in acromegalics following a glucose load. The effect of ingested acetoacetate on fat metabolism in acromegalics is also unknown. The insulin response and its effect on FFA metabolism might, however, be inferred to be normal because of observed post-exercise depression of fat metabolites after a rise in insulin in acromegalic subjects, as described earlier in Chapter 9.

In order to examine the inference of high levels of HGH on insulin dependent changes of fat metabolism during oral glucose and acetoacetate tolerance tests, a number of untreated and treated acromegalic patients were investigated.

10.2 PROCEDURE

Five acromegalic patients (4 women aged 24-57 and one male aged 52) were studied before operation for ablation of their

pituitary tumour. Three of the patients were also studied between 3 and 6 months afterwards. The patients had glucose and acetoacetate tolerance tests on separate occasions. The procedure was the same as that described in Chapter 8 on the responses of patients suffering from hypopituitarism.

The results were compared with those obtained from the same control group used earlier in relation to the hypopituitary patients.

10.3 RESULTS

10.3.1 RESULTS OF GLUCOSE TOLERANCE TESTS (Fig. 10:i, Table 10:i)

Resting levels of glucose were slightly higher in the patients compared with the normal controls (92 mg/100 ml, 81 mg/100 ml) but the differences were not significant. The mean rise in glucose was greater in the acromegalic patients but because of the large spread of values the maximum concentrations at 30 min were not significantly different from the control mean (162 ± 18; 150 ± 8 mg/100 ml). Glucose concentration fell in both groups during the remainder of the investigation. Lactate and pyruvate concentrations were similar throughout the investigation in both groups. Growth hormone levels (Table 10:i) were depressed by glucose in the control subjects but were unaffected in the patients.

The plasma insulin concentrations were slightly higher in the acromegalic patients at rest but not significantly so. However, ingestion of glucose caused a much greater rise in insulin concentration in the patients so that their plasma insulin values were significantly different from thos of the controls at 30 min (112 ± 22; 55 ± 14 u units/ml. $P < 0.01$).

Fig. 10:i Mean changes in blood glucose (mg/100 ml) plasma IRI (μunits/ml), plasma FFA (% resting values) and blood ketone-bodies (% resting values) before and after ingestion of 50 g glucose by 7 control subjects (o — o) and 6 acromegalic patients (■ --- ■). Statistical significance assessed by Mann-Whitney u test.

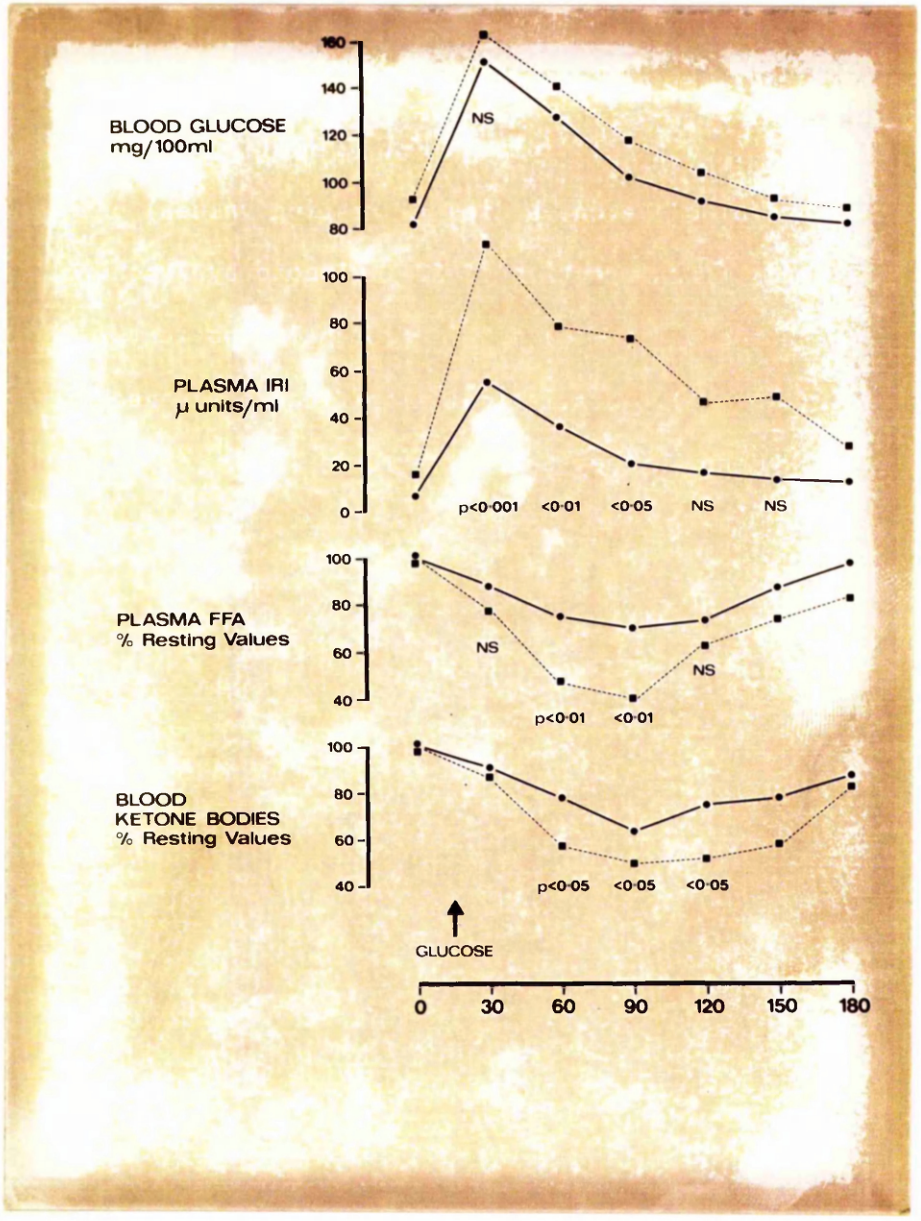

BLOOD GLUCOSE
mg/100ml
160
140
120
100
80
NS
PLASMA IRI
μ units/ml
100
80
60
40
20
0
p<0·001
<0·01
<0·05
NS
NS
PLASMA FFA
% Resting Values
100
80
60
40
NS
NS
p<0·01
<0·01
BLOOD
KETONE BODIES
% Resting Values
100
80
60
40
p<0·05
<0·05
<0·05
GLUCOSE
0
30
60
90
120
150
180

TABLE 10:i Changes in plasma HGH, FFA and blood Ketone-bodies during oral glucose tolerance tests (50g Glucose) in 5 acromegalic patients and 7 control subjects. (HGH estimated in 6 controls only).

	Time (min)	0	30	60	90	120	150	180
Plasma FFA (μequiv/ml)	Controls Mean	0.62	0.53	0.44	0.38	0.43	0.52	0.61
	Patients Mean	0.85	0.61	0.38	0.32	0.50	0.59	0.63
Blood Ketone-Bodies (μ mol/ml)	Controls Mean	0.76	0.68	0.59	0.48	0.52	0.56	0.67
	Patients Mean	0.85	0.74	0.52	0.38	0.47	0.52	0.72
Plasma HGH (μ units/ml)	Controls Mean	8.2	7.4	5.3	6.1	6.2	7.6	9.3
	Patients Mean	116	121	109	98	114	102	105

The plasma concentrations fell in parallel with the glucose levels in both groups but the values in the patients remained significantly different from control values at 60 min ($P < 0.01$) and 90 min ($P < 0.05$).

The acromegalic patients had higher resting levels of plasma FFA and blood ketone-bodies, although not significantly so. In both groups of subjects ingestion of glucose caused a fall in the level of plasma FFA and blood ketone-bodies but the percentage fall was greater in the patients. The percentage of resting FFA values at 60 and 90 min were significantly lower in the patients (60 min - 48 ± 6% SEM; 75 ± % SEM; P 0.01; 90 min 41 ± 9% SEM; 70 ± 7% SEM; $P < 0.01$). During the subsequent rise to normal levels the values were similar in both groups. The depression in ketone-bodies was significantly greater in the patients at 60 min (55 ± 9% SEM; 36 ± 8% SEM; $P < 0.05$) and 90 min (47 ± 8% SEM; 30 ± 6% SEM; $P < 0.05$) and 120 min (42 ± 8% SEM; 27.2 ± 8% SEM; $P < 0.05$).

10.3.2 RESULTS OF ACETOACETATE TOLERANCE TESTS (Fig.10:ii, Table 10:ii)

The increases in blood ketone-body concentration were similar in both groups following ingestion of acetoacetate. There was a slightly faster fall in values of blood ketone-bodies in the patients between 60 and 120 min but the differeme did not achieve significance.

Blood glucose levels were slightly higher in the patients than in the control subjects (89 ± 6 mg/100 ml; 81 ± 4 (SEM) mg/100 ml) but ingestion of acetoacetate caused a smaller depression of glucose levels in the patients to that observed in the controls. However

Fig. 10:ii Mean changes in blood ketone-bodies (μmol/ml), blood glucose (% resting values), plasma FFA (% resting values), plasma IRI (μunits/ml) before and after ingestion of 200 ml 0.4 M acetoacetate by 7 control subjects (⊙ —— ⊙) and 5 acromegalic patients (□ ——□). (N.B. individual results in 3 patients for plasma IRI).

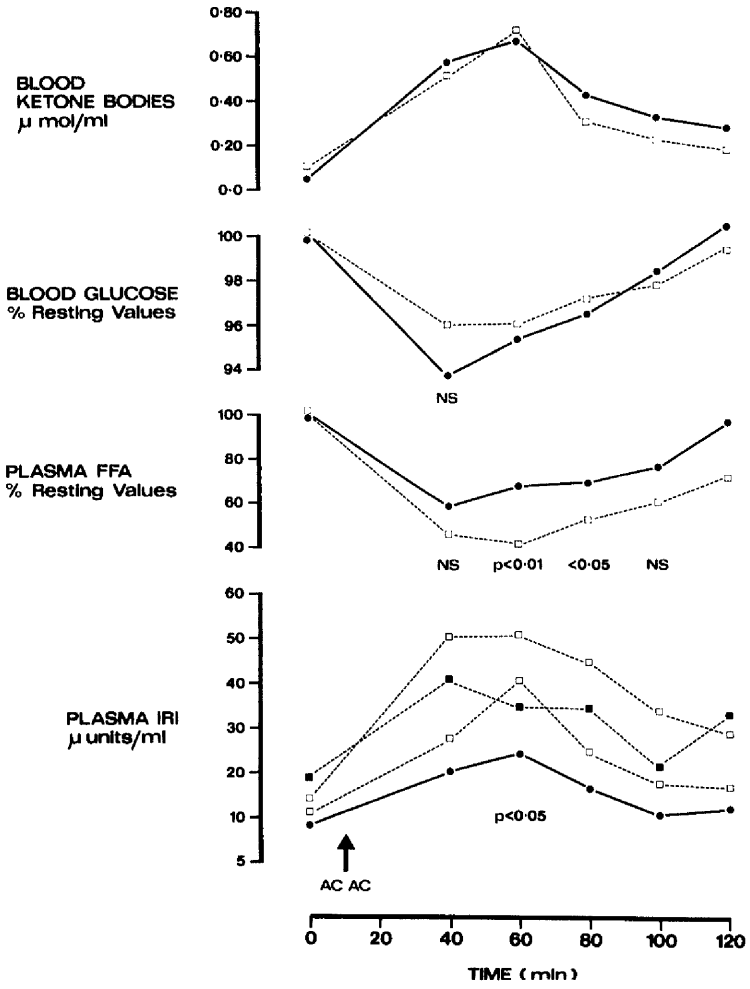

BLOOD
KETONE BODIES
μ mol/ml
0·80
0·60
0·40
0·20
0·0
BLOOD GLUCOSE
% Resting Values
100
98
96
94
NS
PLASMA FFA
% Resting Values
100
80
60
40
NS
p<0·01
<0·05
NS
PLASMA IRI
μ units/ml
60
50
40
30
20
10
5
p<0·05
AC AC
0
20
40
60
80
100
120
TIME (min)

Table 10.ii

Changes in blood glucose and plasma FFA in 5 acromegalic patients and 7 control subjects during oral acetoacetate tolerance tests (200 ml 0.4M)

	Time (min)	0	40	60	80	100	100
Blood Glucose (mg/100 ml)	Control Mean ± SEM	81 ± 6	76 ± 5	77 ± 5	78 ± 4	79 ± 7	82 ± 9
	Patients Mean ± SEM	89 ± 4	82 ± 5	83 ± 6	86 ± 8	87 ± 7	88 ± 6
Plasma F.F.A. (μequiv/ml)	Control Mean ± SEM	0.62 ±0.05	0.36 ±0.07	0.42 ±0.07	0.43 ±0.09	0.47 ±0.10	0.59 ±0.06
	Patients Mean ± SEM	0.88 ±0.10	0.42 ±0.06	0.36 ±0.12	0.47 ±0.09	0.53 ±0.12	0.62 ±0.04

the mean values in the two groups were not significantly different at any time (Table 10:ii).

Plasma FFA levels when expressed as % of resting values were depressed to a greater extent by ingestion of acetoacetate in the acromegalic patients compared with the controls. The difference was most marked 60 min after taking the acetoacetate (patients 40.5 $\pm$ 7% SEM, controls 68 $\pm$ 11% SEM, $P < 0.01$). Although levels rose to normal values in both groups from 60 min onwards the difference in the depression of FFA levels remained significant ($P < 0.05$) at 80 min.

Plasma HGH concentration was substantially unchanged in either group during the period of the investigation.

Plasma insulin levels were measured in 4 controls and 3 patients only. Resting levels of insulin were higher in the patients (mean 13 μ units/ml) than in the controls (mean 8 $\pm$ 4 μ units/ml). In both groups insulin levels rose after ingestion of acetoacetate but the rise was greater in the acromegalic patients. The mean values at 60 min were significantly greater in the patients compared with the controls ($P < 0.05$, Mann Whitney u Test). Insulin levels fell in both groups to resting levels during the remainder of the investigation.

10.3.3 EFFECT OF TREATMENT OF ACROMEGALY BY CRYOSTATIC ABLATION OF PITUITARY TUMOUR ON GLUCOSE AND ACETOACETATE TOLERANCE (Table 10:iii)

Two of the patients (women aged 54 and 57) had a reduction of levels of HGH after operation but the plasma HGH concentration was still high (68, 47 μ units/ml). They showed slight depression of HGH levels in glucose tolerance tests and the pattern of metabolite

TABLE 10:iii Effect of cryostatic ablation of pituitary tumour in one patient JB. Changes in glucose, HGH and IRI in Glucose Tolerance Tests (50g orally) and in plasma IRI and FFA in Acetoacetate Tolerance Tests (200ml 0.4M AcAc orally) before and three months after operation.

Glucose Tolerance Test	0 min		30 min		60 min		90 min		120 min		180 min	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Glucose mg/100 ml	88	76	151	144	132	127	110	108	100	105	93	87
Plasma HGH (μ units/ml)	68	7	71	6	64	4	76	4	71	7	57	9
Plasma IRI (μ units/ml)	18	11	91	44	97	39	48	23	31	18	20	9

Acetoacetate Tolerance Test	0 min		40 min		60 min		80 min		100 min		120 min	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Plasma IRI (μ units/ml)	14	9	28	9	40	14	24	11	16	8	15	10
Plasma FFA (μequiv/ml)	0.94	0.82	0.80	0.80	0.76	0.78	0.81	0.83	0.80	0.92	0.88	0.85

changes as a result of ingestion of glucose and acetoacetate was essentially similar to the changes observed before operation.

In one of the patients, a young woman aged 24 (JB) a remarkable clinical improvement was shown after operation and resting plasma HGH fell from 66 $\pm$ 7 μ units/ml (measured on three occasions) before to 8 $\pm$ 1.6 μ units/ml (measured on three occasions) after operation. She had undergone exercise tests and both glucose and acetoacetate tolerance tests before and operation. In all of these tests carried out three to four months post-operatively there was a change in the responses of hormones and metabolites to the normal pattern. The most important results from the glucose and acetoacetate tolerance tests are presented in table 10:iii.

10.4 DISCUSSION

The observations of impaired glucose metabolism and exaggerated insulin secretion following ingestion of glucose in acromegaly are similar to those previously reported elsewhere (Ikkos et al., 1962; Karam et al., 1963). The observation of increased insulin secretion following ingestion of acetoacetate by the acromegalic subjects has not previously been reported. But some caution must be exercised in the interpretation of the results since the values for patients and controls shown in Figs. 10:i and 10:ii are compilations from two different assay methods which have previously shown differential systemic errors. However, comparison of the results from the two patients and two control subjects in whom the insulin was assayed by the same method shows nevertheless a greater increase of plasma IRI in the acromegalic subjects. The characteristic insulin resistance of acromegaly is further demonstrated by the slightly smaller

though not substantially significant depression of glucose by acetoacetate compared to control values.

The present results indicate that after taking glucose and acetoacetate the insulin-mediated depression of plasma level of FFA and ketone-body production occurs despite high levels of HGH in acromegalic patients. In fact the greater depression of FFA and ketone-body concentrations when considered as a percentage of the resting levels appears to have been greater in the acromegalic subjects.

This may be related to the greater observed insulin release after taking glucose and acetoacetate.

These observed differences between the acromegalic subjects and the controls seem to depend upon the presence of high levels of HGH and are not necessarily related to the clinical condition of the patient. This is shown by examination of the different results from the two patients investigated post-operatively who had high levels of HGH and in the single patient with low levels of HGH after operation.

The present results amplify the conclusions which were arrived at in the discussion of the post-exercise changes and their differences in acromegalic and normal subjects. There appears to be a large stimulatory effect of fat metabolites on insulin release in acromegaly which is independent of glucose and is not inhibited by elevated levels of HGH. The subsequent effect of insulin in depressing lipolysis and also stimulating lipogenesis, perhaps by the divertion of ketone-bodies into fatty acid synthesis, might

explain the lack of post-exercise ketosis in acromegalic subjects.

10.5 SUMMARY

1. Four patients with active acromegaly were studied during oral glucose and acetoacetate tolerance tests. Three of the patients were also studied after cryogenic ablation of their pituitary tumour. All results were compared with figures drawn from studies in normal controls.

2. Ingested glucose and acetoacetate caused a greater elevation of insulin in the acromegalic patients than in the controls. High levels of HGH inhibited the insulin-induced uptake of glucose in the patients but there was a greater fall in levels of fat metabolites in the patients. The patients who had been successfully treated showed changes similar to those in the controls.

3. It is concluded that the effect of insulin on re-esterification of fatty acids is exaggerated in acromegalic subjects despite their resistance to the action of insulin on uptake of glucose.

CHAPTER 11

CHANGES IN BLOOD METABOLITE CONCENTRATIONS WITH MODERATE EXERCISE IN PATIENTS SUFFERING FROM MUSCULAR DYSTROPHY

11.1 INTRODUCTION

Most of the biochemical studies of muscle diseases have concentrated on the degenerative changes in structural and functional proteins in muscle. However, recently there has been a growing awareness that a true understanding of the causes of the primary myopathies must include results from studies of intermediate carbohydrate and fat metabolism (Pennington, 1969). A growing number of studies of altered fat and carbohydrate metabolism in progressive muscular dystrophy have been reported (see for example Moser, Sluga & Lujf, 1970; Strickland, Lin & Hudson, 1970; Takagi, Shimada & Mozai, 1970; Salmon, Esiri & Ruderman, 1971) but there has been surprisingly little information derived from studies of exercise in patients suffering from muscle diseases.

The work described in the preceding chapters has produced information on muscle energy metabolism in normal subjects or in patients suffering from endocrine disease. It thus provides indices of normal muscle metabolism with which to compare the possibly altered metabolic patterns in muscle disease. The work described in the following chapter was undertaken to provide basic information on metabolic changes during and after exercise in patients with progressive muscular disease.

11.2 PROCEDURE

Six patients were studied. Their details are given in Table 11:i. The investigation was explained to the patients and their consent obtained. The patients fasted for 15 hr

Table 11:i Details of Patients with Myopathy

Patient	Sex	Age (yr)	Height (cm)	Weight (kg)	Average Work Load (kpm/min)	Serum Enzymes Increased	Diagnosis
L.B.	M	59	168	69.0	150	Yes	Limb girdle dystrophy
G.G.	M	50	162	68.5	500	"	Limb girdle dystrophy
D.R.	M	48	178	70.2	500	"	Limb girdle dystrophy
D.S.	M	64	174	89.4	300	"	Peroneal muscular atrophy
J.T.	M	44	169	67.2	600	"	Limb girdle dystrophy
E.W.	F	30	172	45.4	300	"	Distal myopathy

before the exercise, which was carried out in the morning. None of the patients had taken drugs for the period of their fast. They had catheters placed in an arm vein for blood sampling during the investigation. In two of the patients (D.S. and W.A.) respiratory gas analysis was carried out during the investigation. These measurements were kindly performed by Dr. Sheila Jennett. All patients had their heart rates recorded by a continuous e.c.g. The patients exercised on a bicycle ergometer for 20 min at average work loads between 150 and 600 kpm/min. These work loads were adjusted so that the patients showed an increase of heart rate to 150 beats/min after 5 min exercise.

Blood samples were taken at rest, throughout the period of exercise at 5 min intervals and afterwards at 15 - 30 min intervals for 90 min. Analysis was carried out for metabolites and hormones as described in Appendix I. The results were compared with those of 5 male control subjects (aged 26-58) available from previous studies.

11.3 RESULTS

11.3.1 WORK LOAD AND HEART RATE DURING EXERCISE

The patients had resting heart rates in the normal range. The work load was adjusted to give a heart rate of 150 after 5 min of exercise and the loads ranged from 150 kpm/min to 600 kpm/min. In all patients the heart rate continued to rise and reached values of between 158 - 182 at the end of 20 min. The heart rates during exercise were similar to those in normal subjects exercising at 600-800 kpm/min (see Chapter 1 & 4).

11.3.2 METABOLIC CHANGES (Table 11:ii)

The results for patients L.B., C.G., D.R. & J.T. have been averaged since all these patients suffered from limb girdle dystrophy. Results for D.S. and E.W. are presented separately in the tables as their results often differed from those of the four limb girdle patients.

(a) Blood glucose: (Table 11:ii(a)) Resting levels of blood glucose were higher in the patients than in the controls but the mean value in the four patients with limb girdle dystrophy was only marginally significantly different ($P < 0.1$). There was a greater rise in glucose values during exercise in the limb girdle patients and in E.W. than in the control subjects. In D.S. the glucose showed no consistent change except to apparently rise after exercise.

(b) Blood lactate and pyruvate: (Table 11:ii(b)) All patients had higher resting lactate concentrations but pyruvate was significantly higher only in the four patients with limb girdle dystrophy. D.S. had a very low resting pyruvate concentration and the values rose by a small amount only during exercise. In the other patients lactate and pyruvate concentrations were generally higher during exercise and at 15 min afterwards than in the control group.

(c) Blood glycerol and plasma FFA: (Table 11:ii(b)) Blood glycerol and plasma FFA concentrations were normal at rest in D.S. and in the patients with limb girdle dystrophy. In E.W., however, the glycerol was much lower than in the controls although the FFA levels were only slightly lower.

Table III(a)

Metabolic changes during and after exercise in patients with muscular dystrophy and in controls.

	Subjects	Rest	Exercise				Post - Exercise			
			5 min	10 min	15 min	20 min	+15 min	+30 min	+60 min	+90 min
Blood Glucose (mg/100 ml)	Limb-Girdle (4)	88 ±6	87 ±8	91 ±5	98 ±6	102 ±10	97 ±9	89 ±5	94 ±4	93 ±4
	D.S.	100	94	94	111	94	119	123	122	109
	E.W.	92	102	104	106	98	94	102	87	85
	Control (mean ± SEM)	81 ±7	82 ±8	85 ±6	86 ±9	84 ±7	89 ±4	91 ±6	87 ±7	84 ±9
Blood Lactate (μ mol/ml)	Limb-Girdle (4)	0.720 ±0.103	3.17 ±0.68	4.85 ±1.11	5.27 ±1.42	6.61 ±1.50	4.08 ±1.12	3.07 ±0.97	1.97 ±0.062	0.91 ±0.07
	D.S.	0.83	2.89	3.51	3.23	3.94	3.28	2.52	3.05	2.54
	E.W.	1.10	2.50	3.75	4.45	5.80	4.20	3.20	2.81	0.92
	Control (mean ± SEM)	0.691 ±0.108	0.970 ±0.221	1.14 ±0.27	2.86 ±0.82	2.83 ±0.72	1.68 ±0.42	1.02 ±0.31	0.78 ±0.10	0.62 ±0.09
Blood Pyruvate (μ mol/ml)	Limb-Girdle (4)	0.091 ±0.031	0.087 ±0.018	0.144 ±0.012	0.103 ±0.009	0.252 ±0.014	0.225 ±0.112	0.192 ±0.087	0.102 ±0.042	0.051 ±0.027
	D.S.	0.016	0.025	0.053	0.030	0.030	0.076	0.075	0.019	0.021
	E.W.	0.052	0.047	0.093	0.110	0.121	0.131	0.099	0.042	0.061
	Control (mean ± SEM)	0.043 ±0.021	0.048 ±0.014	0.056 ±0.017	0.167 ±0.022	0.107 ±0.009	0.094 ±0.016	0.088 ±0.012	0.046 ±0.008	0.058 ±0.011

Table 11: ii(b)

	Subjects	Rest	Exercise				Post - Exercise			
			5 min	10 min	15 min	20 min	+15 min	+30 min	+60 min	+90 min
Blood Glycerol (μ mol/ml)	Limb-Girdle (4)	0.056 ±0.009	0.048 ±0.007	0.052 ±0.012	0.075 ±0.021	0.031 ±0.013	0.051 ±0.022	0.062 ±0.017	0.048 ±0.031	0.045 ±0.021
	D.S.	0.063	0.084	0.041	0.139	0.124	0.164	0.160	0.118	0.053
	E.W.	0.012	0.019	0.028	0.022	0.084	0.062	0.083	0.071	0.048
	Control (mean ± SEM)	0.072 ±0.011	0.082 ±0.021	0.092 ±0.014	0.114 ±0.010	0.087 ±0.016	0.088 ±0.018	0.096 ±0.022	0.081 ±0.016	0.062 ±0.012
Plasma F.F.A. (μ equiv/ml)	Limb-Girdle (4)	0.882 ±0.103	0.827 ±0.073	0.877 ±0.102	0.903 ±0.094	0.971 ±0.142	0.992 ±0.117	0.863 ±0.098	0.784 ±0.111	0.775 ±0.056
	D.S.	0.992	0.994	0.897	1.030	1.180	1.240	0.970	1.020	0.910
	E.W.	0.784	0.951	1.022	1.015	1.080	1.190	1.000	0.890	0.952
	Control (mean ± SEM)	0.855 ±0.123	0.913 ±0.121	1.041 ±0.106	1.033 ±0.097	0.998 ±0.141	0.928 ±0.188	0.888 ±0.200	0.798 ±0.104	0.867 ±0.094
Blood Ketone Bodies	Limb-Girdle (4)	0.098 ±0.018	0.055 ±0.022	0.103 ±0.031	0.104 ±0.041	0.090 ±0.027	0.085 ±0.019	0.146 ±0.029	0.118 ±0.034	0.097 ±0.024
	D.S.	0.174	0.216	0.208	0.181	0.221	0.271	0.274	0.320	0.287
	E.W.	0.083	0.079	0.102	0.092	0.102	0.115	0.180	0.172	0.148
	Control (mean ± SEM)	0.060 ±0.010	0.058 ±0.009	0.074 ±0.019	0.082 ±0.013	0.091 ±0.019	0.089 ±0.038	0.098 ±0.021	0.115 ±0.027	0.172 ±0.041

Although the glycerol levels in the patients rose during exercise the blood concentrations were much lower than in the control subjects. The changes in plasma FFA were, however, more similar in the patients and in the control group. D.S. had levels of plasma FFA which were consistently higher than the control mean but within the control range.

(d) Blood ketone-bodies (3-hydroxybutyrate and acetoacetate): (Table 11:ii(b)) The resting values of blood ketone-bodies were slightly elevated in all patients compared to the control subjects. The changes during and after exercise were variable in the patients. The four limb girdle dystrophy patients and patient E.W. showed little change during exercise but there was a rise of between 50-70% 30 min afterwards. The levels then fell again to resting values in the succeeding hour. Thus they showed an early post-exercise maximum in the level of ketone-bodies compared to the later rise in the control subjects. The total production of blood ketones were, however, similar to the changes in the control group. D.S. had high resting values (0.174 μ mol/ml) and the level rose immediately during exercise and remained elevated throughout. There was a further small rise in the blood concentration 60 min after exercise. The values in D.S. were at all times much greater than those of the control subjects.

11.3.3 INSULIN AND GROWTH HORMONE (Table 11:iii)

(a) Plasma IRI: Resting concentrations of insulin were low in E.W. and high in D.S. In the four patients with limb girdle dystrophy the values were similar to those in the controls. This pattern remained throughout the exercise

Table 13iii

Hormone changes during and after exercise in patients with muscular dystrophy and in controls.

	Subjects	Exercise					Post - Exercise			
		Rest	5 min	10 min	15 min	20 min	+15 min	+30 min	+60 min	+90 min
Plasma IRI (μ units/ml)	Limb Girdle (4)	12.2 ±3.0	12.0 ±2.6	11.9 ±1.8	11.7 ±4.1	10.0 ±2.6	11.5 ±2.8	13.0 ±3.2	12.8 ±3.1	11.8 ±2.7
	D.S.	13.1	13.0	12.0	11.0	11.5	12.6	12.0	13.0	10.5
	E.W.	8.0	7.5	5.5	6.5	5.5	6.5	7.5	9.0	8.5
	Control (mean ± SEM)	10.5 ±3.1	9.8 ±2.2	8.4 ±1.8	7.5 ±2.4	7.2 ±3.0	8.8 ±1.7	9.4 ±4.1	11.2 ±2.2	11.5 ±1.8
Plasma HCH (μ units/ml)	Limb Girdle (4)	12.4 ±5.2	17.6 ±6.6	22.5 ±7.1	26.0 ±9.7	25.0 ±8.1	20.0 ±4.4	14.4 ±3.0	12.8 ±2.2	9.8 ±6.1
	D.S.	12.0	15.5	18.5	28.0	29.0	27.5	20.0	18.5	13.5
	E.W.	13.0	24.0	29.0	33.0	42.0	29.0	19.5	21.5	15.1
	Control (mean ± SEM)	8.7 ±2.8	12.4 ±3.6	14.2 ±4.0	16.1 ±5.2	17.8 ±4.8	15.6 ±3.0	12.8 ±5.2	10.2 ±1.8	9.1 ±3.7

and post-exercise periods. E.W. showed only a slight fall in insulin levels during exercise but in the other patients the concentration fell about 15%, similar to the change in the control subjects. There was no difference in the rate of return of insulin levels to normal between the patients and the controls and in neither group was there an overshoot above resting levels.

(b) Plasma HGH: Growth hormone levels were higher at rest in the patients than in the controls (12.6 $\pm$ 4 μ units/ml; 8.7 $\pm$ 2.8 μ units/ml) and rose to a greater concentration after 20 min of exercise (36 $\pm$ 9.7 μ units/ml; 17.8 $\pm$ 5.9 μ units/ml).

11.4 DISCUSSION

The results are difficult to assess because of variations between the patients in the severity and nature of their disease and also because of the different work rates they achieved during exercise. Judged from the heart rates they worked at a similar load to each other and to the controls but it is doubtful if an accurate assessment of relative work load can be made from heart rate changes during leg exercise by patients with muscular weakness. However, the patients felt that they had worked hard and the changes in heart rate and metabolites suggest that they worked at least as hard as the controls, although it is not possible to judge whether or not they exercised nearer to their limit than the controls. The factors which limit the performance of exercise are more likely to be muscular in the patients and cardiovascular and respiratory in the controls.

Blood glucose concentrations rose more in the patients, except for D.S., and since the rise has been shown to be correlated with work load (Wahren et al., 1971) in normal subjects this may be evidence that the patients did work relatively harder or that they were more stressed during exercise. Progressive muscular dystrophy is associated with decreased glucose tolerance (Tyler & Perkoff, 1951) and the greater rise might be due to lower peripheral uptake of glucose.

The most striking differences between the patients and the controls were in the changes in levels of lactate and pyruvate and glycerol during exercise. The greater build-up of lactate and pyruvate suggests that the patients were unable to efficiently oxidise carbohydrate during exercise. There is evidence of increased glycolysis distinct from the lactate production of hypoxia in genetically dystrophic hamsters (Dhalla, Fedelsova & Toffler, 1972). The higher levels of lactate and pyruvate both at rest and during exercise may have inhibited lipolysis in adipose tissue and thus depressed the blood concentration of glycerol. There is also evidence for the diversion of glucose from normal pathways of metabolism towards the build-up of triglyceride in dystrophic muscle (Ellis, Strickland & Eccleston, 1970).

The high FFA-glycerol ratio in the patients does not necessarily conflict with this suggestion since the FFA levels in the patients' blood might reflect a poor uptake of circulating fat as fuel. Nevertheless, Fredholm (1970) has suggested that lactate depresses release of FFA from adipose tissue by increasing re-esterification with newly synthesised

α-glycerophosphate and thus glycerol freed during lipolysis continues to be released. This would be in conflict with the present explanation. Although high blood lactate concentrations are seen with exercise in unfit subjects, the pattern of metabolic response usually includes post-exercise ketosis (Johnson et al., 1969). This suggests that the metabolic pattern shown in the patients does not represent a situation of extreme unfitness.

A lower usage of FFA for fuel during exercise in the patients and only partial oxidation in liver might explain the slightly higher levels of blood ketone-bodies during exercise. A decreased utilisation of ketone-bodies by muscle has been postulated as an explanation of ketonaemia by Takagi and co-workers (1970) and Salmon and co-workers (1971). The latter workers reported a hyperketonaemic patient with abnormal lipid accumulation in muscle and mitochondrial inclusion bodies. One male patient with extraocular myopathy was shown to be ketonaemic at rest and during exercise during routine investigation at the University of Glasgow Department of Neurology (Dr. R.H. Johnson & Mr. W.R. Sulaiman, personal communication). On biopsy of muscle this patient also showed evidence of mitochondrial abnormalities with inclusion bodies and lipid deposition. Although the ketonaemia in these patients was greater than in those at present reported, the present findings add weight to the possibility of an impairment of mitochondrial fat oxidation in muscle disorders.

Observations of blood concentrations of metabolites have the disadvantage that they reflect changes in both muscle and

liver metabolism thus a rise in the concentration of one metabolite may be due to decreased utilisation by muscle or liver or increased production by one or other tissue. Recently an atraumatic percutaneous needle biopsy technique has been developed for study of skeletal muscle (Edwards, 1971). This provides enough tissue for light and electron microscopy, and histochemical and biochemical studies of muscle tissue from the same sample may also be possible. The application of these techniques in conjunction with investigation of blood changes would provide much more information on the biochemical processes underlying the changes observed in the present study. Of particular importance would be the study of skeletal muscle concentration of key enzymes in the control of fat and carbohydrate metabolism.

The greater levels in the patients of HGH during and after exercise might be due to greater exercise stress. The HGH levels are, however, raised at rest in the patients; perhaps in response to an increase in protein breakdown and the observed increase might be an exaggerated response after potentiation. Raised levels of HGH at rest have also been seen in patients with Duchenne-type dystrophy (Takagi et al., 1970). The greater increase in HGH during exercise would not help to explain the greater increases in lactate and the apparently deficient glycerol rise; in fact the rise of HGH during exercise has been linked to increased lipolysis (see Chapters 2, 3, 4, 7, & 9).

The metabolic measurements at rest and during exercise in the patients investigated suggests that they have a low

oxidative capacity for carbohydrate metabolism. Although lipolysis is apparently depressed, nevertheless, the subsequent utilisation of circulating fatty acids for fuel appears to be less than in normal control subjects.

11.5 SUMMARY

1. Metabolic changes in six patients with progressive muscular dystrophy were studied during and after exercise between 150 and 600 kpm/min. The results were compared with those of five matched control subjects.

2. Levels of lactate and pyruvate were higher and glycerol lower in the patients during exercise. Changes in plasma FFA and ketone-bodies were similar in both groups.

3. Plasma insulin levels were similar in both groups but plasma HGH levels were elevated at rest and during exercise in the patients.

4. It is suggested that the patients have a poor capacity for oxidative metabolism of stored carbohydrate in muscle. Although lipolysis appears to be impaired the decrease in utilisation of circulating fatty acids results in normal levels of plasma FFA.

5. These observations imply that observations of metabolic changes in muscle during exercise by patients with muscle disorders could be rewarding and require further study.

CHAPTER 12

SUGGESTIONS FOR FURTHER STUDY

In the course of the work carried out for these studies on metabolic and hormonal changes with exercise a variety of new problems have been identified. In many areas of current interest there is a lack of precise information and the currently available data may be variously interpreted.

Results presented in Chapters 1, 2, 3 and 4 demonstrate that large quantities of fatty acids may be mobilised into the circulation during exercise. These studies showed a greater mobilisation of fat in trained subjects during exercise, indicated by the quantities of glycerol released into the blood. This cannot be due to changes in hormonal control since the size and direction of changes of growth hormone, insulin and catecholamines during exercise in trained subjects are all such as to lessen lipolysis in them compared to untrained subjects (see Chapters 2, 3, 4, 7 & 9). FFA release from adipose tissue has been reported to be inhibited by high lactate levels in dogs (Fredholm, 1970; Fredholm, 1972) and the large increases of lactate during exercise by untrained subjects might have this effect. However, Fredholm reports that lactate depresses only the release into the blood of FFA and that lipolysis, indicated by glycerol release, is unaffected. If this is so then the observed situation in trained subjects of high blood glycerol together with low FFA concentrations can not be easily explained on the basis of low lactate production since lactate and FFA levels are higher and glycerol lower in the untrained subjects (see Chapters 2, 3 & 4)

The detailed action of hormones in these metabolic changes remains uncertain. Many of the anomalies, such as apparent increased uptake of glucose during exercise (Wahren et al.,1970)

with decreased plasma insulin levels might be explained by increased sensitivity of exercising muscle to hormones (Nikkilä et al., 1968; Riddle et al., 1972). Other explanations might take account of the possibility that skeletal muscles could themselves act as 'endocrine' glands by locally releasing hormones previously taken up from the circulation. This has been shown to be so for heart muscle and insulin (Wahlqvist et al., 1972a) but not demonstrated for working skeletal muscle.

Many of these problems can be approached by a study of arterial and venous blood concentrations of metabolites and hormones in conjunction with techniques for muscle blood flow. However, the study of body fluids as an indication of the metabolism of perfused organs in living man has several disadvantages. It is difficult to be certain that the concentrations of metabolites in venous blood give reliable information on changes in a single muscle group as they probably reflect metabolism from other organs (drained by the venous return). Blood flow and perfusion data are essential to studies of uptake and production of metabolites in a dynamic situation. These factors make experimental design difficult and investigatory procedure complex. Recently an atraumatic percutaneous needle biopsy technique has been developed for study of skeletal muscle in man (Edwards, 1971). This may provide sufficient tissue for the biochemical estimation of a limited number of enzymes and metabolites. Relatively little work has yet been done on cellular contents of fat and carbohydrate metabolites in working skeletal muscle and the techniques offer opportunites to match changes in blood and muscle in a dynamic situation.

The studies on the metabolic effects of fitness described in Chapters 1 - 4 also demonstrate that the FFA mobilised during exercise are apparently more efficiently oxidised in trained athletes. In untrained individuals, however, incomplete oxidation causes a considerable rise in blood ketone-bodies after exercise. At present the explanation of the observed changes depends upon a more efficient utilisation during exercise of fatty acids in trained muscle compared to untrained muscle. Thus after exercise a considerable amount of mobilised fatty acids remain in the circulation of untrained subjects and these are partially oxidised to ketone-bodies in liver. A number of problems arise from this interpretation in the light of the findings presented in this thesis. First, there is the very much greater mobilisation of fat with exercise in trained subjects judged by the rise in glycerol levels and yet plasma FFA levels are only partially elevated compared to the rise which occurs in untrained subjects. This would mean that the increase in fat-oxidative capacity is very great indeed, greater for example than the changes resulting in lower levels of lactate and pyruvate in athletes. What are the mechanisms for this? Are enzymes of fatty acid oxidation in muscle more active? Are they more efficiently controlled? Are different metabolic pathways utilised? Some evidence of increased fat and ketone-body oxidation capacity linked to higher enzyme activity has been found in investigations of training in rats (Molé, Oscai & Holloszy, 1971; Winder, Baldwin & Holloszy, 1973). The greater levels of activity of enzymes of ketone-body utilisation in muscle from trained

animals suggests that the lower levels of post-exercise ketosis in athletically fit individuals may be partly due to efficient fat and ketone-body oxidation by muscle after exercise as well as during exercise. Since FFA uptake by muscle continues after exercise in untrained individuals (Pruett, 1970c) the corollary of this is that some proportion of the post-exercise rise in blood ketone-body concentration is due to production of ketones not by liver but by muscle. This possibility is supported by the finding of negative a-v difference for 3-hydroxybutyrate, which has been demonstrated in exercising arm muscle (Hagenfeldt & Wahren, 1968).

As well as the continued study of normal muscle biochemistry and physiology, these techniques may be fruitfully applied to the study of muscle disease. In particular, the correlation of changes in skeletal muscle with changes of metabolites in blood observed in patients suffering from lassitude and muscular dystrophy would be of great importance (see Chapters 6 &11). The development of specific biochemical and histochemical techniques for diagnostic purposes might then become possible.

APPENDIX I

EXERCISE TESTING, HEART RATE, METABOLITES IN BLOOD, CORTISOL, HGH AND IRI

AI. METHODS

AI.1 EXERCISE TESTING

An electrical, variable load Elema-Schönander Ergometer, type EM 369, was used in all the exercise tests except for some of the investigations described in Chapters 5 and 9, in which friction-resistance machines were used.

Heart rate was routinely recorded by e.c.g. using lead system II, with leads applied to the chest wall.

Chapter 2 contains details of respiratory measurements made during the investigations described there.

AI.2 BLOOD SAMPLING

Blood was taken either by venepuncture or via an indwelling catheter fitted with a tap previously placed in an arm vein at the antecubital fossa. The dead space in the catheter was filled with 0.9% saline between samples and this was withdrawn and discarded before blood samples were taken.

Blood (14 ml) was withdrawn without stasis into unheparinised 20 ml syringes and 4 ml immediately deproteinised by addition to 5 ml of ice cold 10% (w/v) perchloric acid in preweighed tubes. The tubes were thoroughly shaken. The remainder of the blood was transferred to Lithium heparin tubes and gently mixed. All samples were stored on ice until they could be centrifuged at 3000 rpm for 20 min. After spinning the tubes containing deproteinised blood were reweighed to determine the sample volume. Both the supernatant deproteinised extract and the separated plasma in the heparin tubes were transferred into clean tubes and analysed immediately or frozen at -20^{o}C until analysis could be carried out. Estimation of blood glucose, lactate, pyruvate, glycerol, acetoacetate and 3-hydroxybutyrate

were carried out on the neutralised, deproteinised extract. Plasma was used for estimation of FFA, IRI, HGH and cortisol. Additional blood was also taken (20 ml) in those investigations in which catecholamines were measured (Chapter 4).

AI.3 TREATMENT OF DEPROTEINISED EXTRACT

The volumes of sample extracts were measured in graduated glass tubes and recorded. Two drops of universal indicator were added and the extracts neutralised to about pH 6.5 by addition of 20% (w/v) potassium hydroxide. The neutralised samples were allowed to stand at 4°C for 30 min to complete precipitation of potassium perchlorate and were then centrifuged for 5 min at 3000 rpm. The neutral volume was recorded and the supernatant transferred to a clean plastic sample tube. Analysis for acetoacetate was always carried out rapidly after neutralisation because of spontaneous decarboxylation to acetone in neutral solution.

AI.4 ANALYSIS OF ACETOACETATE, 3-HYDROXYBUTYRATE, GLYCEROL, LACTATE, PYRUVATE AND GLUCOSE

Enzymatic methods were used for the estimation of the metabolites in the deproteinised extracts from blood. Enzymes, co-enzymes and standard substrates were purchased from The Boehringer Corporation (London) Ltd. All other chemicals used were of analytical grade and were purchased from B.D.H. (Poole) Ltd or Hopkins & Williams Ltd.

All of the methods used depend on the absorption of light at 340 nm by reduced nicotinamide adenine dinucleotide (NADH) and the lack of absorption for the oxidised form at the same wavelength. Any dehydrogenase reaction in which NAD is reduced or NADH oxidised can be measured by recording the increase or decrease of the

absorbance at 340 nm, if the stoichiometry of the reaction and the molar absorbance coefficient of NADH are known.

For example, lactate dehydrogenase catalyses the reversible reaction

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \underset{\text{pH 6.9}}{\rightleftharpoons} \text{Lactate} + \text{NAD}^+$$

The amount of NADH oxidised is the same as that of pyruvate reduced. The change in absorbance A, is related to the concentration c, of NADH, its molar extinction coefficient ε and the light-path of the cuvette d; e.g.

$$A = \varepsilon \times c \times d$$

For a 1 cm cuvette

$$c = \frac{A}{\varepsilon}$$

and at 340 nm $\varepsilon = 6.22 \times 10^3$

If the assay volume is V, the sample volume is v and the sample dilution factor is F, this becomes

$$c = \frac{A}{6.22} \times \frac{V}{v} \times F = \mu\text{moles NADH/ml blood} \equiv \mu\text{ moles pyruvate/ml blood}$$

By changing the conditions of assay such as buffer pH, NAD or NADH present and the presence or absence of hydrazine hydrate to trap ketones as the hydrazones, the basic assay system was used to measure pyruvate and lactate (Hohorst, Kreutz & Bücher, 1959), and acetoacetate and 3-hydroxybutyrate (Williamson, Mellanby & Krebs, 1962). Glycerol was measured by linking the glycerokinase reaction to the formation of ATP in the pyruvic kinase reaction and measuring the appearance of pyruvate using NADH and lactate dehydrogenase (Kreutz, 1962).

Glucose was measured by a linked reaction involving oxidation by glucose oxidase and the reduction of molecular oxygen to peroxide. The peroxidase reaction can be followed by the use of an organic dye as a redox acceptor in a colorimetric procedure (Werner, Rey & Wielinger, 1970). The method was automated using a peristaltic pump, turntable sampler and filter colorimeter.

AI.5 ANALYSIS OF PLASMA FFA

Plasma FFA were extracted into chloroform by the method of Itaya and Ui (1965) and the extracted fatty acids determined as the copper soap with diethyldithiocarbamate in an automated modification of their colorimetric procedure (Dalton & Kowalski, 1967).

AI.6 ANALYSIS OF PLASMA CORTISOL

The method of Mattingly (1962) was modified for a plasma sample of 0.4 ml. After extraction from plasma with methylene chloride and phase separation with siliconised filter paper the samples were acidified with sulphuric acid/ethanol and the fluorescence spectra determined in an Aminco-Bowman S.P.F. using 170 µl capacity microcells. Measurements were taken at 468/520 nm in accordance with the recommendations of Usui, Kawamoto and Shimao (1970) to avoid nonspecific fluorescence at shorter wavelengths.

AI.7 RADIO-IMMUNOASSAY FOR HUMAN GROWTH HORMONE (HGH)

HGH measurements reported in Chapters 9 and 10 were kindly made by Dr. M.H.C. Webster using the method of Hunter and Greenwood (1962). Measurements of HGH reported in Chapter 4 were kindly made by Mr. W.R. Sulaiman. Other measurements were made by the author.

The method used for iodination of HGH was that of Greenwood, Hunter and Glover (1963) with modifications due to Sulaiman (1973). Briefly, the method is the reaction between ^{125}I and buffered HGH in the presence of chloramine-T. The reaction is stopped by sodium metabisulphite and the iodinated hormone is applied to a Sephadex G-50 (Pharmacia) column. The hormone is eluted with 5 ml fractions of barbitone buffer.

AI.7.1 DOUBLE-ANTIBODY ASSAY OF HGH

The method used was a double-antibody procedure (Morgan & Lazarow, 1963) modified by Sulaiman (1973). MRC standard A calibrated against WHO reference preparations was used. The scheme of the assay was as follows:-

HGH Assay Scheme

1. Add 1.0 ml HGH standard (0.25 - 4.0 ng) or unknown plasma sample diluted 1:10 to 50 µl anti-HGH serum (1:8000).
2. Mix and incubate 4°C for 3 days.
3. Add 50 ul of ^{125}I HGH (2 µg/ml).
4. Mix and incubate 4°C for 3 days.
5. Add 50 ul rabbit anti-HGH serum (DR 16 Wellcome, diluted 1:8000).
6. Add 50 ul donkey anti-rabbit serum (Wellcome, diluted 1:5)
7. Mix and incubate 4°C for 24 hr.
8. Add 1.0 ml phosphate /BSA buffer, centrifuge at 4°C , 2300 rpm for 30 min.
9. Decant supernatant and count precipitate for 2 min in gamma counter.
10. Prepare calibration curve of log % bound HGH vs ng HGH in standard.

AI.8 RADIO-IMMUNOASSAY FOR IMMUNOREACTIVE INSULIN (IRI)

Dr. M.H.C. Webster kindly performed some of the assays reported in Chapters 9 and 10. Some measurements reported in those Chapters and in Chapter 4 were also made by Mr. W.R. Sulaiman. All other assays were performed by the author.

The method for insulin assay was similar to that for HGH except that instead of separating free and bound insulin after reaction with the first anti-body by the use of a second, albumin coated charcoal is used to adsorb free insulin (Herbert, Lau, Gottlieb & Bleicher, 1955; Hunter & Ganguli, 1971).

No iodination of native insulin was necessary as this was purchased from the Radiochemical Centre, Amersnam. Wellcome Insulin (MR 71) was used for standards.

The scheme for insulin assay was as follows:-

Scheme for IRI Assay

1. 1 ml standard IRI (1 - 20 μ units/ml) or unknown plasma sample (diluted 1:10) added to 50 μl Guinea-pig anti porcine insulin antiserum (MT 41, Wellcome).
2. Mix and incubate 4°C for 3 days.
3. Add 50 μl ^{125}I insulin (1 μg/ml).
4. Mix and incubate 4°C for 3 days.
5. Add 1.0 ml charcoal slurry (1g Norit NK suspended in 0.3 g BSA/100 ml in phosphate buffer pH 7.4).
6. Mix for 1 min, centrifuge at 4°C 3000 rpm for 30 min. Decant supernatant, wipe inside to remove excess free insulin and count in gamma counter for 2 min.
7. Prepare standard curve as before for HGH.

APPENDIX II

MODIFICATION OF EXISTING TECHNIQUES FOR SEPARATION AND DIFFERENTIAL ASSAY OF ADRENALINE AND NORADRENALINE IN BLOOD

AII METHODS

AII.1 INTRODUCTION

Adrenaline (A) and noradrenaline (NA) are present in blood in very small amounts. Various authors have reported widely different values for plasma concentration at rest, as shown below.

Table AII.i Catecholamines in Human Plasma at Rest

Adrenaline ng/l plasma	Noradrenaline ng/l plasma	Author
0	300 ± 110	Häggendal (1968)
280 ± 60	790 ± 40	Carruthers, Taggart, Conway, Bates & Somerville (1970)
220 ± 40	580 ± 110	Weil-Malherbe, quoted in Udenfriend (1962)
66 ± 13	174 ± 24	Renzini, Brunori & Valori (1970)
50 ± 30	200 ± 80	Engleman & Portnoy (1970)

The large variation in the reported figures reflects the highly complicated and difficult nature of present methods for isolation and measurement of physiological amounts of catecholamines in plasma. This section deals with the modification and characterisation of a technique (Renzini, Brunori & Valori, 1970) for analysis of small amounts of catecholamines using a two-step chromatographic isolation and fluorimetric measurement of the pure amines.

AII.2 PREVIOUS PROCEDURES FOR THE PURIFICATION OF CATECHOLAMINES FROM PLASMA

Organic solvent extraction procedures for catecholamines are restricted in their usefulness to the analysis of tissues and

adsorption techniques alone are adequate for purification from body fluids. Chemical analysis of catecholamines first became practical when Shaw (1938) described the pH-dependent adsorption of the amines on to freshly prepared aluminium hydroxide. Lund (1949) showed that the adsorption was more efficient with alumina and he adopted column adsorption with alumina at pH 8.6. The catecholamines could be eluted from the columns with dilute acid and Lund found a 92% recovery in his procedure using 0.2N acetic acid. The basic techniques using alumina have been modified by a large number of workers and purification by both column and batch methods under a variety of conditions of pH, ionic strength, prior deproteinisation and temperature have been reported, (for review see Weil-Malherbe, 1972). Although the mechanism of adsorption of catecholamines on to alumina remains unknown, recovery is improved if commercial alumina is treated with hot 2N HCl, washed repeatedly in distilled water and activated by heating at 300°C (Weil-Malherbe & Bone, 1952; Anton & Sayre, 1963). Drying at 200°C is only about 10% less efficient but 100°C gives poorly adsorbent substances (Weil-Malherbe, 1972).

Catechol compounds can be bound in ionic form at acid pH but only in the absence of phosphate and other ions, the amines are susceptible to oxidation and there is a danger of elution during later washing with water (Drell, 1970). At pH 8.5,however, ions do not interfere and the amines are protected from oxidation once adsorbed (Weil-Malherbe, 1970). Most procedures describe the addition of Na_2 - EDTA and sodium metabisulphite to further protect against oxidation in the presence of heavy metals.

Cation exchange resins have also been used for catecholamine extractions. Strong sulphonic acid resins have been used by some

workers (Bertler, Carlson & Rosengren, 1958; Hüggendal, 1968) and weak carboxylic acid types by others (Kirshner & Goodall, 1957; Barchas, Erdelyi & Angwin, 1972). Strong resins bind the amines very effectively but need high concentrations of mineral acids for elution, often causing oxidation of the amines. Weakly acidic resins are less effective in adsorption but elution may be performed with a small volume of weak acid, e.g. 1N acetic acid.

For a weak carboxylic acid resin, effective at about pH 6, sample electrolyte concentration must be reduced to a minimum because of the competitive effects of cations. This means amines may not be purified from urine and perchloric acid extracts from blood and tissues without prior treatment to reduce their ionic strength.

Catecholamines form a strongly acidic complex with boric acid (Mattock, Wilson & Heacock, 1966) which protects them to some extent from oxidation in air and which may be used to elute the amines adsorbed on to a weakly acidic resin.

The techniques of chromatography using both alumina and carboxylic acid resins in columns and subsequent elution with a small volume of boric acid were combined by Valori and co-workers (Valori, Renzini, Brunori, Porcellati & Corea, 1969) to produce a reportedly highly purified and concentrated plasma extract of catecholamines.

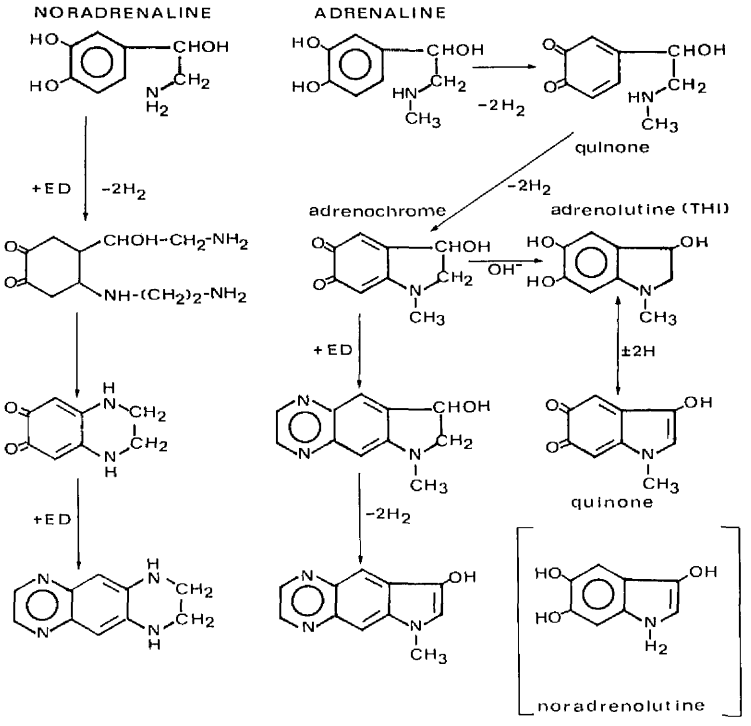
AII.3 TYPES OF PROCEDURES FOR THE FLUORIMETRIC ESTIMATION OF CATECHOLAMINES

In the presence of molecular oxygen catecholamines may be spontaneously oxidised through a quinone intermediate to a pink

substance, the aminochrome (see Fig. AII:i). This rearranges by cyclisation in alkaline solution to form a trihydroxyindole - the aminolutine. Aminolutine is relatively highly fluorescent compared to the non-specific native fluorescence of catecholamines attributable to their phenolic structure. The increased fluorescence forms the basis of biochemical procedures for measuring adrenaline and noradrenaline in body fluids. The oxidation products are similar for adrenaline and noradrenaline except that with noradrenaline the product lacks the methyl group on the indole nitrogen. The aminolutine is very unstable in alkaline solution unless protected from oxidation by a suitable reducing agent. The trihydroxyindole (THI) method has been applied in a variety of modifications, all of which attempt to measure the fluorescence of the THI product at the limits of manipulative skill and instrument sensitivity (for review see Udenfriend, 1962;1969; Weil-Malherbe, 1972). Most of the modifications are concerned with increasing specificity and lowering the reagent-blank often by extended purification of samples and reagents. Recently, however, Valori and co-workers have managed to increase the fluorescence of the lutine and render it much more stable by the use of 2, 3 mercapto-propanol in formalin and acidifying after the alkali rearrangement (Valori, Brunori, Renzini & Corea, 1970).

A second reaction widely used in fluorimetric assay of catecholamines is the condensation of oxidised catechols with ethylenediamine (see Fig. AII:i). Adrenaline reacts with one molecule of ethylenediamine and maintains its N-methylethanolamine side-chain but noradrenaline reacts with two molecules of the reagent and loses the side-chain to yield two products, different from each other and from the adrenaline product in their fluorescence characteristics. Catechol, dihydroxymandelic acid and dopamine also

Fig. AII:i Scheme of condensation of catecholamines with ethylenediamine and oxidation of catecholamines to form trihydroxyindole derivatives.


NORADRENALINE
ADRENALINE
+ED
-2H2
adrenochrome
quinone
adrenolutine (THI)
OH-
±2H
noradrenolutine

form fluorescent compounds similar to those of noradrenaline. The method has been continuously developed since being first described (Weil-Malherbe & Bone, 1954) but besides including an organic extraction step to separate the fluorescent product, the method is less specific than the THI procedure, is light sensitive and even greater care must be taken in the purification of reagents.

Recently methods have been described for assay of adrenaline and noradrenaline which are based on enzymatic incorporation of a labelled methyl donor group with the catecholamine molecule (Saelens, Schüen & Kovacsics, 1967; Engleman & Portnoy, 1970). The method of Saelens and co-workers is of use for noradrenaline only since it depends on N-methylation. However, Engleman and Portnoy use catechol-O-methyltransferase to 3-methylate both amines, which may eventually be separated by thin-layer and other types of chromatography. Nevertheless, these procedures are extremely complicated and time consuming and the specificity of the methods depends not upon the enzyme, which methylates any catechol compound, but on the efficiency of tedious solvent extraction and chromatographic procedures.

It seems, therefore, that the assay of noradrenaline and adrenaline by the THI method remains the most convenient method for rapid analysis of plasma catecholamines.

AII.4 CATECHOLAMINE ASSAY IN PLASMA BY THE METHOD OF RENZINI, BUNORI, & VALORI (1970)

As originally reported the method consists of alumina adsorption of catecholamines on columns and purifications of the eluate with Amberlite CG50. Elution was performed with boric acid (Valori et al., 1969). The eluate was assayed for catecholamines using a modified THI method which used a novel reducing agent,

2, 3 dimercaptopropanol (BAL) in formalin and adjustment to acid pH to stabilise the fluorophors after alkaline rearrangement. (Valori et al., 1970) The procedures were combined and later reported together with the description of a microtechnique for the THI reaction (Renzini et al., 1970). These methods formed the basic of the modified and improved procedures described below.

The scheme of the reported method was as follows:-

1. 10-20 ml blood chilled and heparinised; plasma separated and 100 mg sodium metabisulphite added.

2. Plasma, 500 mg alumina, 5 ml 5% EDTA, 200 µl 10% sodium metabisulphite stirred together in 100 ml beaker by magnetic stirrer and pH adjusted to 8.4 with 0.5N sodium carbonate. Stirring continued for 7 min.

3. Supernatant decanted and alumina transferred to 7 mm id column and washed in with 10 ml of water followed by 1 ml of 0.05N perchloric acid (PCA). Elution with 2 x 2.5 ml 0.05N PCA. 100 µl 5% EDTA, 100 µl 0.1% ascorbic acid added to stabilise.

4. Eluate adjusted to pH 6.0 - 6.2 with 0.2N carbonate and poured into column (2.7 mm id) of 150 mg Amberlite CG50 as Na^+ form. Column washed with 5 ml water and then with 100 µl 2/3 M boric acid. Elution with 500 µl 2/3 M boric acid.

5. For THI reaction 100 µl eluate mixed with 10 µl 0.01N HCl, 5 µl $CuCl_2$ $2H_2O$ (0.002%). Oxidation is performed by mixing in 5 µl 0.25% $K_3Fe(CN)_6$ with a vortex mixer. Exactly 3 min later 5 µl of 10% BAL in 25% formalin added to stop reaction and 20 µl of 10N NaOH added within 20 sec. After 5 min 15 µl glacial acetic acid is added to adjust to pH 5.3.

6. Samples assayed with internal standards (10 µg of each amine in 10 µl 0.01N HCl) and faded sample blanks are prepared by adding 20 µl 10N NaOH 3 min after oxidation, followed 10 min later by BAL formalin and glacial acetic acid. External samples and reagent blank prepared in 2/3 M boric adjusted to pH 7.

7. Fluorescence read on Aminco-Bowman SPF in 170 µl cuvettes at 415/500 and 460/500 for differential discrimination of the amines.

8. Amount of amines determined by solving:

$$F_1 = yN_1 + xA_1$$

$$F_2 = yN_2 + xA_2$$

x, y = ng adrenaline, noradrenaline in sample

F_1, F_2 = blank corrected fluorescence at 415/500 and 460/500.

A_1, A_2 = Fluorescence/ng of each

N_1, N_2 = external standard amine at 415/500 and 460/500

$$y = \frac{F_1 A_2/A_1 - F_2}{N_1 A_2/A_1 - N_2} \quad ; \quad x = \frac{F_2 - yN_2}{A_2}$$

AII.5 MODIFIED EXTRACTION OF AMINES FROM PLASMA

When this procedure was followed the results were very poor. Recoveries varied from sample to sample in a random fashion. Furthermore, the fluorescence of internal standards which had been added to sample eluates was usually greatly reduced compared to external standards and was often abolished completely. This suggested that the sample eluates contained some substance which inhibited THI reaction.

The following problems were also identified:

(a) The magnetic stirring-bug ground up the alumina particles so that they failed to settle and caused losses of adsorbed amines. These small particles also clogged the columns and flow through the columns was slowed.

(b) Small strands of protein became attached to the alumina and clogged the columns.

(c) Great skill was required to transfer the alumina quantitatively to the columns without losses on glassware.

(d) Column flow-rate was difficult to adjust and varied from sample to sample depending on unforseeable variables e.g. amount of protein precipitated during storage of plasma.

(e) The pH of the PCA eluate from the alumina varied unpredictably even after care was taken to control the flow rates. However, once the alumina eluate was obtained, the column chromatography using Amberlite CG50 proceeded well. It was decided, therefore, to substitute elution from 500 mg batches by mixing alumina in tubes in place of the column elution procedure. Glass centrifuge tubes (26 mm od, 30 ml capacity) fitted with snap-on caps were used to hold the samples. In order to avoid grinding the alumina, the sample pH was adjusted during rapid stirring without alumina, which was then added at pH 8.5. Since the alumina is slightly acid a small readjustment of pH was necessary. The capped tubes could then be rotated on a Matburn cell-suspension mixer to allow complete adsorption on to the alumina. After allowing the alumina to settle, the sample supernatant was aspirated to waste, the sample washed with water and 0.05N perchloric acid was added to the tube. The tube was then recapped and rotated on the Matburn mixer to elute the catecholamines.

To discover the optimum conditions for adsorption and elution of the catecholamines a number of experiments were performed.

AII.6 CONDITIONS FOR ADSORPTION AND ELUTION OF CATECHOLAMINES USING 500mg BATCHES OF ALUMINA

Recoveries of catecholamines added to expired plasma were determined both fluorimetrically and by the use of radiolabelled catecholamines (DL adrenaline -7-H3 HCl 13Ci/mmol, DL noradrenaline (methylene -C14) DL-bitartrate 56 mCi/mmol, The Radiochemical Centre, Amersham). Aliquots of 0.1-2 uCi of 3H adrenaline and/or 0.01- 0.1 uCi of 14C-noradrenaline were added to the samples. Recovery was determined by comparing original counts with counts in aliquots removed from the processed samples and added to 5 ml Insta-gel (Packard). A Packard Tri-carb model 3220 liquid scintillation spectrometer was set to count 3H and 14C simultaneously (3H, Red channel 90%, 50-250; efficiency 10%; Green channel 9%, 250-1000; efficiency 28.8%; all counts 10 min).

AII.6.1 Effect of duration of adsorption and extraction on Matburn Mixer

Table AII:ii Recovery of labelled ^{14}C noradrenaline adsorbed by mixing with 500 mg alumina at pH 8.5 for 2, 4, 6, 8, and 10 min followed by 16 min extraction with 5 ml 0.05N PCA.

Time of Adsorption	% Recovery (mean ± SD)	n	P
2 min	48.6 ± 6.3%	4	P < 0.01
4 min	57.3 ± 1.3%	4	
6 min	56.1 ± 0.9%	4	
8 min	54.7 ± 1.7%	4	NS
10 min	55.2 ± 3.7%	4	

These results suggest that noradrenaline is rapidly adsorbed onto alumina at pH 8.5 and further mixing does not increase recovery.

Table AII:iii Recovery of ^{14}C labelled noradrenaline adsorbed by 4 min mixing after extraction for 4, 8, 12 and 16 min with 5 ml of 0.05N PCA.

Time of Elution	% Recovery (mean ± SD)	n	P
4 min	53.7 ± 4.11%	4	
8 min	54.2 ± 4.1%	4	NS
12 min	54.8 ± 5.2%	4	
16 min	57.3 ± 1.0%	4	

These results suggest that 4 min elution is adequate.

N.B. These results suggest a rather lower recovery of amines from the alumina than is currently reported. However, the alumina used was prepared by heating at 180°C only. When alumina heated to 300°C is used recovery at the PCA elution is 70-90% depending on the time elapsed since the alumina was activated.

AII.6.2 Optimal strength and volume of PCA eluant.

Recovery of aliquots of 25 µg noradrenaline and 25 µg adrenaline added together to 10 ml expired plasma and eluted by varying volumes and strengths of PCA was determined by fluorimetric assay after Amberlite chromatography. Because of instrument difficulties at this stage in the method development, especially of light scatter, the recoveries are given in terms of arbitrary fluorescence units at 420 nm, since only poor discrimination of the amines was then possible.

Table AII:iv Influence of concentration and volume of PCA used to extract amines from alumina.

PCA Volume	PCA Normality	Fluorescence (arbitrary units) means ± SD	n
4 ml	0.0425 N	88.8 ± 5.6	4
4 ml	0.050 N	89.0 ± 8.4	4
5 ml	0.0425 N	93.5 ± 1.7	4
5 ml	0.050 N	98.3 ± 4.0	4
6 ml	0.0425 N	86.0 ± 6.1	4
6 ml	0.050 N	59.5 ± 38.2	4

Since the fluorescence for similar quantities of mixed external standards at 420 nm was 240 units the recoveries were about 35-40% except for the 6 ml 0.05 N sample, for which the recovery was about 25%.

These results showed that elution with 5 ml of 0.05 N PCA was most efficient. Furthermore, if greater amounts of PCA were used the efficiency fell sharply, a surprising result. However, when the pH of all the Amberlite eluates were measured and small but significant differences were noted from a mean of about pH 7.43 for the sample treated with 4 ml of 0.0425 N PCA to pH 7.18 for the samples treated with 6 ml of 0.05 N PCA. The relationship between the pH of the eluant from the Amberlite and its amine content, and possible effects on the THI reaction were unknown and it was decided to examine these factors.

AII.7 RECOVERY OF CATECHOLAMINES AFTER AMBERLITE CHROMATOGRAPHY OF PCA ELUATE

Samples containing both radio-labelled (3H 0.1 μCi, ^{14}C 0.01 μCi) and unlabelled catecholamines (100 ng of noradrenaline) were extracted

from plasma using alumina and the extracts were applied to the Amberlite (0.2 ml moist resin, 150 mg) columns. The columns were washed with water, 100 µl 2/3 M boric acid and eluted with 500 µl 2/3 M boric acid. Samples were taken for radiometric and fluorimetric analysis. The fluorimetric assay was run with 100 µl aliquots of the eluate (i.e. expected to contain 20 ng of amine approx.) with and without internal standard and the results were compared with external standards.

Table AII:v Recoveries in eluate from Amberlite CG50

Radiometric assay % recoveries (mean ± SD) 16 samples		Values found after fluorimetric assay Expected values at least 20 ngNA 16 samples	
NA ^{14}C	A ^{3}H	No Internal Standard	20 ng Internal Standard
76.6 ± 2.1	63.5 ± 1.7	2.7 ± 2.3 ng (i.e. 13.5%)	8.4 ± 5.6 ng (i.e. ⩽20ng)

These results indicated that even though the recovery of very small quantities of radio-labelled amines was of a workable order, some substance in the eluate was interfering with the fluorophors formed by the THI reaction.

This was then tested by examining the recovery in serial fractions eluted from Amberlite of labelled and unlabelled amines. These were added to 5 ml PCA eluates from 12 blank plasma (6 A, 6 NA) samples and applied to the Amberlite columns (0.2 ml, 150 mg). Fractions of 100 µl of boric acid eluate were collected. The same fractions from different columns were pooled to give two series of pooled fractions of 600 µl each, one series containing adrenaline and the other noradrenaline. Aliquots from each fraction were taken for radiometric and fluorimetric measurements.

Table AII:vi Recovery of catecholamines after fractional elution from Amberlite

Fraction (µl)	% Total Counts ^{3}H Adrenaline	% Total Counts ^{14}C Noradrenaline	% Recovery by Fluorimetry of added Adrenaline	% Recovery by Fluorimetry of added Noradrenaline	Does Fraction Decrease Fluorescence of added Internal Standard?	pH of Fraction
0-100	0.2	0.06	0	0	Yes	8.25
100-200	2.2	1.4	0	0	Yes	8.20
200-300	37.6	25.5	38.1	24.2	No	7.30
300-400	39.3	38.4	39.0	38.2	No	7.00
400-500	15.4	22.0	16.4	14.1	No	6.95
500-600	3.9	8.8	2.2	5.9	No	6.90
700-800	1.3	3.7	0.9	2.7	No	6.90
800-900	0.1	0.4	-	-	No	6.90

These results showed that the first 200 µl of the boric acid e luate from alumina contained a substance which interfered with the T HI reaction. Since these 200 µl did not apparently contain any catecholamines this fraction could be discarded. It seems likely that Na^{+} as sodium borate is washed off in excess in this fraction and i nt erferes with the fluorophor formation in some way. These results partially explained the previous difficulties encountered in obtaining consistent results following Valori's practice, which recommended a pre-elution wash of 100 µl of boric acid. Presumably, depending on variation in the column behaviour, this volume was often sufficient to partially remove bound Na^{+} but occasionally the 500 µl of eluant proper carried most, and subsequent fluorimetric analysis was impossible.

The batch method for adsorption on to alumina, the larger volume pre-elution wash and the addition of radioactive amines for recovery e stimation were incorporated into the modified method. Recovery of e xogenous radio-labelled catecholamines added to expired plasma and p urified by this method is 82 ± 7% for ^{3}H adrenaline and 84 ± 5% for ^{14}C n or adrenaline (42 samples).

A II.8 OTHER MODIFICATIONS TO THE METHOD OF RENZINI ET AL.

(a) In order to use Eppendorf micropipettes and tips, the volumes of acetic acid added to the sample in the THI reaction was increased to 20 µl and the glacial acetic acid diluted by 30%.

(b) Test tubes for the THI reaction were siliconised with 2% dichlorodimethylsilane in carbon tetrachloride so that losses due to tube wetting by the small volumes used (160 µl) were avoided.

(c) To reduce the interference of elastic scatter during spectrophotometric scanning of the excitation spectra of the fluorophors a piece of polaroid film was inserted in the emission monochromator to pass only vertically polarised light. This had little attenuating effect on the fluorescence but reduced scatter considerably, since in our system only horizontally polarised light may be scattered through 90^{o} in the plane of wave propagation (D. Jolly, personal communication).

AII.9 A NOTE ON METHODS OF BLOOD SAMPLING AND TREATMENT OF SAMPLES

Carruthers and co-authors (1970) have drawn attention to the rapid destruction of catecholamines in blood and plasma after sampling and they recommended the immediate separation of plasma from blood cells and platelets by centrifugation for 3 min at 5000 rpm and freezing of plasma by solid CO_2 at once. Decay of catecholamines continues in plasma if the plasma is not frozen. Since this procedure was found to be very cumbersome when serial samples were taken an alternative method was sought to combine practical ease with an acceptably small degree of destruction of catecholamines.

The oxidation of catecholamines by molecular oxygen in the presence of heavy metals may be countered by chelating agents and

reducing agents. However, monoamine oxidase and catechol-O-methyl transferase activity are also present in blood, probably associated with cells and platelets (Carruthers et al., 1970). Monoamine oxidase is inhibited by pargyline and catechol-O-methyl transferase by pyrogallol and these substances have previously been used to protect catecholamines in homogenates of brain tissue (Saelens, et al., 1967).

In order to determine the usefulness of the addition of inhibitors in blood samples, the recovery of added ^{14}C noradrenaline was determined in a series of blood samples (a) immediately separated and frozen after addition of 50 mg EDTA, (b) samples to which 50 mg EDTA, 50 mg metabisulphite and 500 µg pargyline were added and separation delayed at room temperature, and (c) samples transferred to ice cold universal bottles containing EDTA, metabisulphite and pargyline and separation delayed during storage in ice water. All samples were immediately frozen by immersion in isopentane/solid CO_2 after separation by centrifugation at 3000 rpm. Samples were then extracted using alumina and Amberlite CG50.

Table AII:vii Decay of exogenous catecholamines added to whole blood at sampling: effect of pargyline and temperature.

Sample Treatment	Percentage recovery of ^{14}C noradrenaline after delay in separation (mean ± SD)			
	15 min	30 min	60 min	120 min
Whole blood + EDTA 18°C	40 ± 6	38 ± 8	36 ± 6	33 ± 5
Whole blood + EDTA + metabisulphite + pargyline 18°C	55 ± 4	47 ± 3	44 ± 5	41 ± 4
Whole blood into cold tube + EDTA + metabisulphite + pargyline 0°C	59 ± 3	55 ± 4	53 ± 2	52 ± 3

These results demonstrate (a) the presence of metabisulphite and pargyline decrease decay of amines, even at room temperature in whole blood, and (b) storage of blood with inhibitors at 0°C after rapid cooling by delivery into ice cold sample bottle further decreases initial decay and under these conditions blood may safely be kept in ice water for at least one hour before separation. Although there were noticeable differences in the recoveries, which were probably due to the different treatment of the samples, all of these were in the range 40-60%, whereas recovery of added amines from plasma has been about 80%. This discrepancy might have been due to deactivation of alumina. An alternative explanation is that exogenous catecholamines are distributed between the plasma and the cellular elements of the blood, which are subsequently separated by centrifugation. Endogenous catecholamines may also be distributed in this way and this would affect plasma values in situations of changing haemoconcentration.

It appears, therefore, that as a practical procedure the addition of a monoamine oxidase inhibitor in combination with a chelating agent and a reducing agent to ice cold sample tubes of large thermal inertia will decrease the decay of catecholamines in blood samples. The efficacy of pyrogallol as a catechol-O-methyl transferase inhibitor in blood could not be tested using radio-labelled amines since the O-methylated product behaves similarly in the purification procedure. However, the results indicate that it might be beneficial. Tropolones are better inhibitors than pyrogallol and are not deliquescent. They could, therefore, prove more useful practically as catechol-O-methyl transferase inhibitors (Belleau & Burba, 1963). This could be tested by using the THI reaction which distinguishes the catechol and methyl catechol derivatives.

AII.10 SCHEME OF MODIFIED METHOD

AII.10.1 Reagents and glassware

Double distilled water used for all reagents. All glassware steeped in concentrated nitric acid overnight and washed three times with distilled water and oven dried. Test-tubes siliconised with 2% dichlorodimethylsilane in carbontetrachloride. Alumina and Amberlite CG50 prepared by the method of Renzini et al., (1970). Standards made up as 500 µg/ml base from adrenaline bitartrate and noradrenaline bitartrate (Sigma Chemical Company). Working solutions were 1 ng/µl in 0.01N HCl.

AII.10.2 Sampling procedure

A mixed dry powder of sodium metabisulphite 5 g, sodium EDTA 5 g and pargyline 5 mg was prepared (pargyline is poisonous). 100 mg powder added to each 30 ml dry glass universal specimen tube and plastic caps tightly screwed on. Tubes kept in ice before and during use. Centrifuge switched on in advance of collecting samples. 20 ml blood withdrawn through indwelling catheter with minimum suction to avoid haemolysis, transferred to ice cold tube, cap replaced and tube mixed thoroughly without allowing the hand to warm the tube. Kept on ice. Plasma separated by centrifuging for 20 min at 4°C at 3000 rpm. 10 ml plasma poured into plastic tubes and frozen plasma samples stored in deep freeze.

AII.10.3 Extraction of catecholamines - STAGE I

1. Check pH electrode against standard buffers and repeat from time to time during stage I and stage II.
2. Prepare 0.1% ascorbic acid (10 mg/10 ml water).
3. Label tubes and add 5 ml 5% EDTA, 200 µl metabisulphite

freshly made up.

4. Similarly process a plasma blank and recovery standard (radio-labelled, 0.01 μCi ^{14}C NA)

5. Add sample (10 ml) and 5 ml water.

6. Add magnetic bug, adjust pH to 8.4 - 8.5 with 1N carbonate and brisk magnetic stirring. Add alumina (500 mg) check pH, readjust if necessary and remove bug. Put on caps and mix on Matburn for 4 min. Keep pH electrode upright.

7. Allow to settle for 2 min then aspirate supernatant.

8. Wash with 5 ml water by rota mixer for 5 sec, allow to settle and repeat, centrifuge for 2 min.

9. Elute with 5 ml fresh (pH 1.1 - 1.4) 0.05N PCA for 5 min on Matburn.

10. Eluate transferred to 10 ml flask with 100 ul 0.1% ascorbic and 100 μl 5% EDTA. Store at 4°C overnight if necessary.

(Double distilled water throughout)

AII.10.4 Extraction of catecholamines - STAGE II

1. Allow PCA eluate to come to room temperature.

2. Check pH of Amberlite suspension (6.5) from time to time.

3. Wash 4 - 5 ml resin 6 times with water and drain.

4. Set up columns with BAF tips, 0.2 ml of moist resin and wash in with 5 ml water. Remove all air bubbles.

5. Adjust PCA eluate pH to 6.0 - 6.2 with 0.2N carbonate and add to column. Allow column to drain.

6. Wash with 10 ml water.

7. Wash with 200 μl 2/3 M boric and remove last drop with test tube.

8. Elute with 500 μl 2/3 M boric.

9. Keep eluate at 0°C before proceeding.

10. Remove 100 µl for radioassay for recovery.

(Double distilled water throughout)

AII.10.5 Fluorimetric assay of purified catecholamines

1. Prepare BAL reagent: 800 µl 40% formaldehyde, 200 µl BAL, 1 ml water, cover with parafilm and mix periodically for 10-15 min on rotamix or until quite clear (filter if necessary).

2. Make up working standards of 1 µg/ml of base from stock solutions (500 µg/ml 0.1N HCl stable at 2°C 1 month).

3. Make up $K_3Fe(CN)_6$ 0.25% (stable at 2°C for 2-3 weeks).

4. Switch on fluorimeter and plotter following sequence in warm up procedure. Wash and polish microcells. Determine position of minimum scatter for each cell.

5. Prepare fluorophors for samples, standards and blanks (Table AII:viii).

6. After 30 min, run spectra of fluorophors, measure standard and sample deflection, subtract faded blank and solve simultaneous equation (see AII.4)

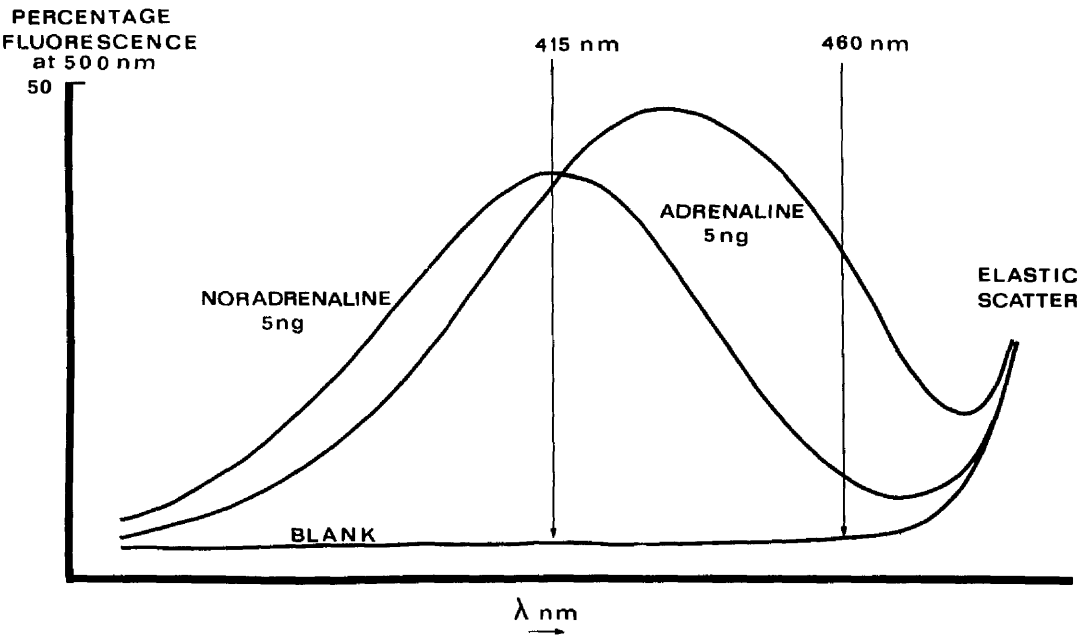
Table AII:viii Schedule of THI reaction

Sample + internal standard faded blank	Sample + internal standard assay	External standard	Reagent blank
100 µl eluate 10 µl A	100 µl eluate 10 µl A	100 µl blank eluate 10 µl 0.01N HCl	100 µl blank eluate 20 µl 0.01N HCl
5 µl Cu'			
* 5 µl K_3Fe			
3' 20 µl NaOh 13' 5 µl BAL) 20 µl HAc)	3' 5 µl BAL) 20 µl NaOH) 8' 20 µl HAc		

* start clock

The fluorescence spectra of the fluorophors of adrenaline and noradrenaline are shown in Fig. AII:i.

Fig. AII:ii Fluorescence spectra of trihydroxyindole derivatives of noradrenaline (noradrenolutine) and adrenaline (adrenolutine). Tracing of original recording; meter multiplier setting 3%; P.M. tube sensitivity 90%.


PERCENTAGE
FLUORESCENCE
at 500 nm
50
415 nm
460 nm
ADRENALINE
5ng
NORADRENALINE
5ng
ELASTIC
SCATTER
BLANK
λ nm

AII.11 PLASMA CATECHOLAMINE CONCENTRATION ESTIMATED BY THE MODIFIED METHOD

Differential assay of adrenaline and noradrenaline was carried out for blood samples from 12 subjects. Ten were normal healthy young men (age 22-33 yr) in whom blood was taken at rest in the sitting position via an indwelling catheter previously placed in an arm vein. Another subject was a patient (age 60 yr) being investigated for autonomic failure producing a disorder of blood pressure regulation. The other sample was taken during operation from the adrenal vein of a patient before removal of a suprarenal pheochromocytoma.

Table AII:ix Plasma catecholamine concentrations

	Adrenaline ng/ml	Noradrenaline ng/ml
Normal subjects (10) (mean values $\pm$ SEM)	78 $\pm$ 17	185 $\pm$ 20
Autonomic Failure (1)	100	210
Pheochromocytoma (1)	-	2000

These resting values are similar to those reported recently by Renzini et al., (1970) and by Engleman and Portnoy (1970).

AII.12 SUMMARY

1. An account is given of various methods for assay of catecholamines in blood plasma and the difficulties commonly encountered are discussed.

2. One method (that of Valori et al) has been extensively examined and systematically modified to provide a relatively rapid, sensitive and accurate assay of plasma catecholamines.

3. Values of adrenaline and noradrenaline in plasma from 10 normal

resting subjects were 78 $\pm$ 17 ng/ml and 185 $\pm$ 20 ng/ml respectively.

4. This method is currently being used for investigations. Results of an investigation using the method are described in C hapter 4, in which it is shown that plasma levels of catecholamines are lower in trained subjects than in untrained individuals at the same relative work level.

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