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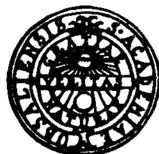
118

SUBSTRATE AND HORMONE INTERRELATIONSHIPS IN HUMAN MYOCARDIAL METABOLISM

by

Mark L. Wahlqvist



Uppsala 1972

From the Department of Geriatrics (Head: Professor L.A. Carlson), University of Uppsala, Uppsala; the King Gustaf Vth Research Institute (Head: Professor G. Birke), Stockholm; and the Department of Clinical Physiology (Head: Professor T. Sjöstrand), Karolinska Hospital, Stockholm, Sweden.

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Uppsala, 1972

the memory of a swedish grandfather

the charity of a chinese wife

the future of an australian son

A thesis based on the following papers:

I. Wahlqvist, M.L., Kaijser, L., Lassers, B.W. and Carlson, L.A. Fatty acid as a determinant of myocardial substrate and oxygen metabolism in man at rest and during prolonged exercise. Acta Med. Scand. (in press).

II. Wahlqvist, M.L., Kaijser, L., Lassers, B.W., Löw, H. and Carlson, L.A. The role of fatty acid and of hormones in the determination of myocardial carbohydrate metabolism in healthy fastimg men. 1972.

III. Wahlqvist M.L., Kaijser, L., Lassers, B.W., Löw, H. and Carlson, L.A. Release of insulin from the human heart. 1972.

IV. Wahlqvist, M.L., Kaijser, L., Lassers, B.W., Löw, H. and Carlson, L.A. Glucocorticoid uptake and release by the human heart: studies at rest, during prolonged exercise and during nicotinic acid infusion. 1972.

V. Wahlqvist, M.L., Rössner, S., Kaijser, L. and Carlson, L.A. Myocardial metabolism during infusions of glucose and a fat emulsion in healthy men: studies at rest and during prolonged exercise. 1972.

VI. Wahlqvist, M.L., Kaijser, L., Eklund, Brita, Rössner, S. and Carlson, L.A. Relationships between myocardial carbohydrate and free fatty acid metabolism in angina pectoris: Studies during atrial pacing and nicotinic acid infusion. 1972.

These papers will be referred to in the text by their Roman numerals as listed above.

INTRODUCTION

Several substrates in the blood are now known to provide energy for the human myocardium. The measurement of arterio-venous concentration differences across the human heart became possible after 1947 with the advent of coronary sinus catheterisation (27, 28). Glucose (4, 29), lactate (4, 29), pyruvate (4, 29) and ketone bodies (5) were soon recognized as substrates for the human heart as had been found for isolated animal hearts (56, 57). Although the work of Bing et al (5) had suggested that fatty acid could be used by the human myocardium, it was Gordon and Cherkes in 1956 who found that free fatty acids (FFA) could be taken up by human heart muscle (30, 31). More recently, endogenous plasma triglycerides have been established as a myocardial fuel in man (12, 43, 44). Some evidence is now available that exogenous plasma triglycerides may also serve as a substrate for the human heart (13).

In addition, the human heart has endogenous stores of glycogen and triglycerides which can apparently be called upon during ischaemia, when the myocardium may produce lactate (54, 55, 58), and during prolonged exercise, when the myocardium may produce glycerol (39, 44).

The literature suggests that glucose (4, 29, 39), lactate (29, 39, 46), pyruvate (29, 39, 46) and FFA (15, 43, 46, 51) are taken up by the human heart in relation to their respective concentrations in arterial blood (56, 57). The relationships between uptakes of different substrates by the isolated perfused heart have been explored in considerable detail (24, 25, 26, 52, 53, 63) and the findings have formed part of the basis for the "glucose-fatty acid cycle" hypothesis of Randle and coworkers (59). It was proposed

that fatty acid was a major determinant of myocardial carbohydrate metabolism and that high plasma FFA concentrations led to low myocardial carbohydrate uptake. For the human heart, however, only a limited amount of information about substrate interrelationships is available (45).

Myocardial oxygen metabolism might be affected by substrate availability as well as by work and other factors (65). It is known that the energy obtained from a given amount of oxygen is less for lipid than for carbohydrate oxidation (74). In the isolated rat heart increased FFA oxidation is accompanied by increased oxygen consumption (17-19) and, in dogs, myocardial oxygen consumption is increased when the plasma FFA concentration is elevated (49). The possibility that FFA might affect myocardial oxygen metabolism in man has not been investigated.

Various hormones could affect myocardial metabolism in man although little is known about what action hormones have at physiological concentrations in healthy individuals (56, 57, 68). The administration of insulin to diabetics does increase myocardial glucose uptake (29).

The heart presumably takes up, and may release, those hormones which affect its metabolism and animal studies show this for catecholamines (10) and thyroid hormones (34).

Most studies of human myocardial metabolism have been carried out with subjects in the fasting state. The fed state is a more variable state. Therefore, those studies of arterial-coronary sinus concentration differences after a meal of carbohydrate (4, 29) or fat (5, 13), or after intravenous glucose injections (4) might over- or under-estimate substrate uptake because of rising or falling arterial substrate concentrations respectively.

The ischaemic heart has an increased requirement for energy produced by anaerobic processes, such as is the case with anaerobic utilization of glucose. If, in the healthy heart, FFA decrease the myocardial uptake of the "anaerobic fuel" glucose (Fig. 1) and carbohydrate metabolism has less of an oxygen requirement than FFA metabolism, it may be of clinical interest to know whether the same applies to the ischaemic heart (42). Once infarcted, the myocardium apparently has decreased oxidative and glycolytic activity and increased hexose monophosphate shunt activity (Fig. 1) (32).

The present thesis examines the following questions concerning myocardial metabolism in man:

(1) The relationships between carbohydrate and FFA metabolism in the healthy heart in both the fasting and parenterally-fed state and in the ischaemic heart of patients with coronary heart disease.

(2) The relationship between myocardial oxygen and FFA metabolism in healthy men.

(3) The possible effects of arterial insulin, growth hormone and glucocorticoid at physiological concentrations on myocardial carbohydrate metabolism in healthy individuals.

(4) The possible uptake and release of the hormones insulin, growth hormone and glucocorticoid by the heart in healthy individuals.

(5) Exogenous plasma triglyceride as a substrate for the heart in healthy subjects given constant infusions of a fat emulsion and glucose solution.

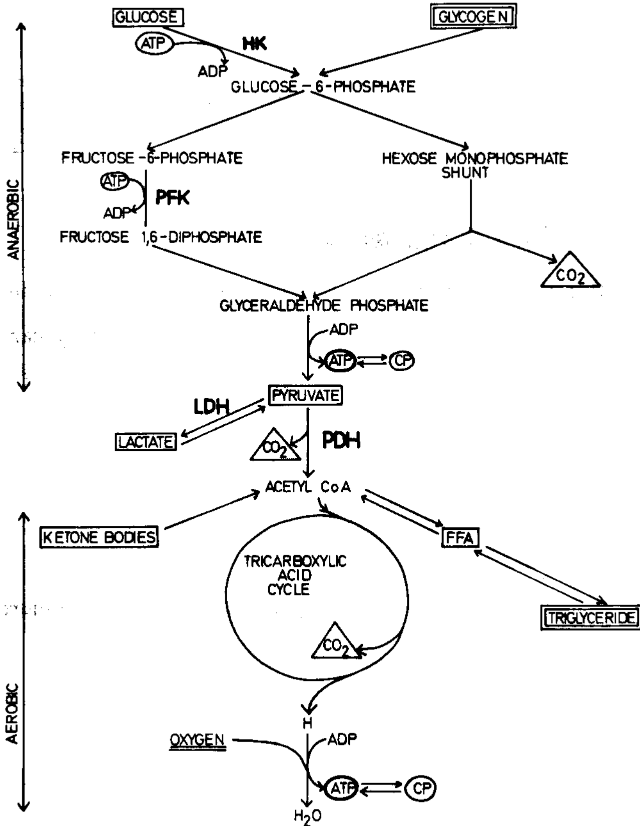

Figure 1: The major steps in the utilization of blood and endogenous substrates for energy metabolism by the myocardium are shown. Blood substrates are enclosed by boxes; endogenous substrates by double boxes; high energy phosphate stores of adenosine triphosphate and creatine phosphate by circles; events tending to increase pH by triangles. HK = hexokinase; PFK = phosphofructokinase; LDH = lactic dehydrogenase; PDH = pyruvate dehydrogenase.

Methods

Subjects

These were either healthy men (Papers I - V) or male sufferers of angina pectoris (Paper VI). The former had no history of cardiovascular or metabolic disease and had a normal resting electrocardiograph (ECG). Where exercise studies were planned the healthy men had normal exercise ECG's. Some of the subjects with coronary insufficiency had had a myocardial infarction, but not less than 5 months before the study; known diabetics were excluded, but no selection was made on the basis of serum lipid values.

Design of Studies

The general plans of the different study types are shown in Figures 1 - 6.

Two days before some studies (indicated in the respective papers) subjects were given an oral dose of iodine in the form of Lugol's solution followed by an intravenous injection of about 6 μCi ^{125}I-albumin. The ^{125}I-albumin was intended to serve as a tracer for plasma protein so that shifts in plasma water could be recognized. This technique was used where the change in concentration of a substrate or hormone from artery to coronary sinus was expected to be small.

Each study was conducted the morning after an overnight fast. Subjects rested, exercised or underwent atrial pacing whilst in the supine position and ECG recordings were made. Catheters were inserted into the right brachial artery and into the coronary sinus percutaneously from a left arm vein for blood sampling. The coronary sinus catheter was made of Teflon with

MYOCARDIAL METABOLISM IN RESTING FASTING MAN

DESIGN OF STUDY

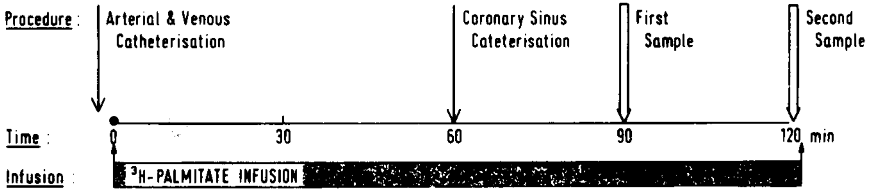

Figure 2: Design of study used in Paper I. Beginning of study was marked by commencement of albumin-bound ^{3}H-palmitate infusion. Paired arterial and coronary sinus blood samples were taken at 90 and 120 minutes.

MYOCARDIAL METABOLISM DURING PROLONGED EXERCISE

DESIGN OF STUDY

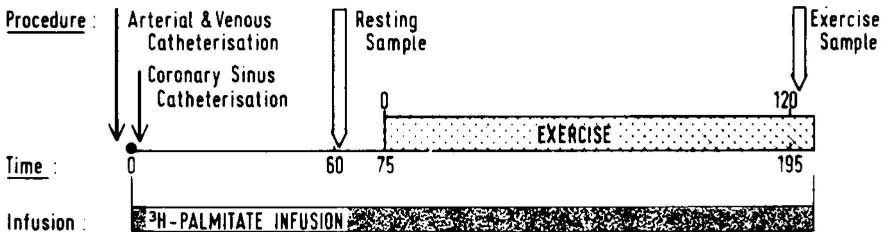

Figure 3: Design of study used in Papers I, II, III and IV. Paired arterial and coronary sinus blood samples were taken after 60 minutes rest and again during the final minutes of prolonged exercise.

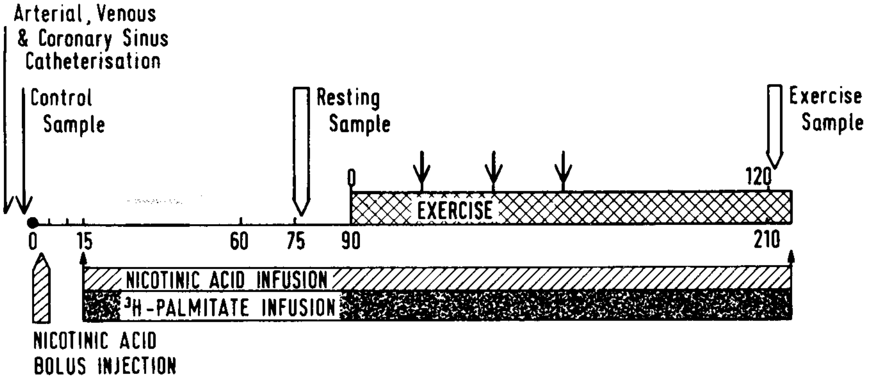

Figure 4: Design of study used in Papers I, II, II and IV. Apart from continuous infusion of nicotinic acid, design is similar to that shown in Figure 3.

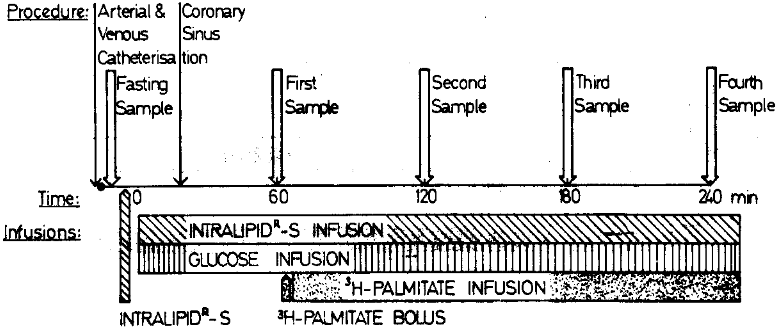

Figure 5: Design of study used in Paper V. Continuous infusions of the fat emulsion Intralipid^{R}-s and glucose solution were given to produce a steady fed state. Paired arterial and coronary sinus samples were taken at 60, 120, 180 and 240 minutes after administration of a priming injection of Intralipid^{R}-S.

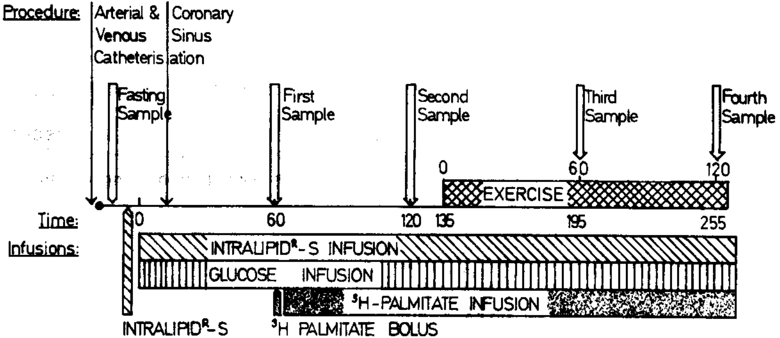

Figure 6: Design of study used in Paper V. It differs from that in Figure 4 in that exercise was begun after 2 hours at rest and continued for up to 2 hours. Paired arterial and coronary sinus samples were taken at rest, at 60 and 120 minutes, and again 60 minutes after the commencement of prolonged exercise and during the final 5 minutes of exercise.

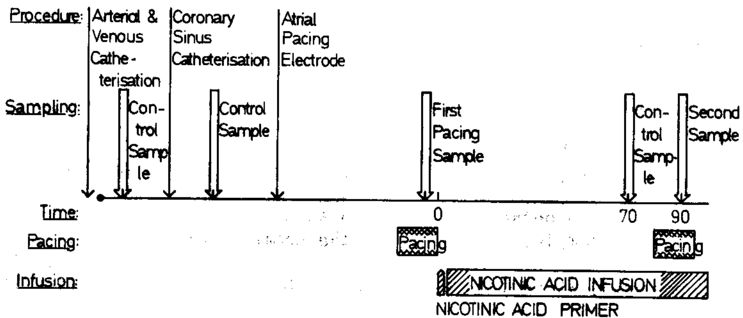

Figure 7: Design of study used in Paper VI. Paired arterial and coronary sinus samples were taken during atrial pacing. For the first pacing, sampling was begun 2 minutes after the commencement of that heart rate at which moderate angina was experienced. In some subjects, nicotinic acid was infused from soon after the first pacing until completion of the second pacing. The second pacing and sampling during the second pacing were conducted in precisely the same way as for the first pacing. If less angina were experienced during the second pacing, pacing was continued at a higher level and this was referred to as "the third pacing".

an internal diameter of 1.55 mm and an external diameter of 2.30 mm and, at the tip, an internal diameter of 1 mm and an external diameter of less than 1.5 mm. It was manipulated into position under fluoroscopic control using an image intensifier and with continuous ECG monitoring. The coronary sinus was recognized as an area which did not give rise to ectopic beats. The edge of the sinus could be felt as the tip of the catheter passed over it. The catheter was introduced 6 - 8 cm beyond the orifice of the sinus and to within 2 - 3 cm of the left heart border. Confirmation that the catheter was with in the sinus was obtained by a comparison of oxygen saturation in the sinus with that in the right atrium. The arterial catheter was kept patent by intermittent flushing with isotonic saline and the coronary sinus catheter by a continuous infusion of 0.5% citrate in isotonic saline at a rate of about 50 ml hourly. Heparin was not administered.

For the infusion of ^{3}H-palmitate, nicotinic acid or fat emulsion, a cannula was inserted percutaneously into a right arm vein. Glucose solution (in combination with ^{3}H-palmitate) was infused through a catheter whose tip was in the superior vena cava; this catheter was also inserted percutaneously into a right arm vein.

Arterial blood samples were taken at the commencement of a study, or before infusions began, as control or fasting samples. Subsequently, simultaneous arterial and coronary sinus samples were drawn as indicated in the figures.

Materials

Palmitic-9, 10- ^{3}H(N) acid (New England Nuclear Corporation) was repurified, sterilised, checked for pyrogenicity and bound to human albumin according to Boberg (6, 7, 43).

^{125}I-albumin was prepared and made available by Prof. G. Birke and Dr L.O. Plantin[*]. Less than one per cent of the iodine was non-protein bound (72).

The fat emulsion used was a modified form of intralipid[R], designated Intralipid[R]-S[xx]. It is an emulsion of soy bean oil (10 g %) and egg yolk phospholipid (1.2 g %) stabilized and made isotonic with 5 g % sorbitol. At infusion rates of less than 100 ml/hour, FFA (1700 µmol/l) and glycerol 800 µmol/l) in the emulsion were infused at about 1 - 2 % of their endogenous turnover rates.

Infusions

Albumin-bound ^{3}H-palmitate was infused at about 0.7 µCi/min as a tracer for plasma free fatty acids and to label endogenous plasma triglyceride (12, 43) (Papers I, II and V). In those studies where the first arterial-coronary sinus sampling was made at 60 minutes rather than 90 minutes, the infusion was preceded by a bolus of about 10 µCi albumin-bound ^{3}H-palmitate to produce rapid labelling of plasma FFA.

A priming dose of 200 mg 5% sodium nicotinate (ACO, Solna, Sweden) was given intravenously before commencement of nicotinate infusions. Infusion rates of 200 mg/hour or 400 mg/hour (Papers I - IV and VI) were used. The latter dose was sufficient to produce consistent lowering of plasma FFA concentration even during prolonged exercise.

Glucose was infused as a 20% solution at rates of 0.31 - 0.32 g/min (Paper V).

Intralipid[R]-S was infused at rates of 0.16 - 0.17 g triglyceride/min.

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[x] King Gustaf V Research Institute, Stockholm, Sweden.

[xx] Dr Ivån Håkansson, AB Vitrum, Stockholm, Sweden, kindly prepared this emulsion.

Prolonged Exercise

Supine leg exercise was performed at a fixed work load on a cycle ergometer (37) after observations at rest. The work load (kpm/min) was chosen two days before the study at the time of an exercise test. It was 50% of the load which produced a heart rate of 170/min after 6 minutes of exercise (W_{170}) (64, 71). It was intended that exercise should continue for 2 hours, a duration of work tolerated by most healthy subjects at the load used (1). Several subjects stopped before 2 hours on account of exhaustion. The last exercise sampling was made during the final five minutes of exercise. Heart rates were read from continuously recorded ECG's.

Atrial Pacing

Right atrial pacing was used as a way of producing angina pectoris in patients with coronary insufficiency. Angina produced in this way is more suitable for the study of myocardial metabolism than is exertional angina since the metabolic and haemodynamic changes of exercise are avoided (54, 55). Also, since haemodynamic correlates of angina seem quite reproducible at a second pacing (3, 66), the same might be the case for metabolic correlates.

A bipolar electrode catheter (United States Catheter and Instrument Corp, Glens Falls, N.Y.) was inserted percutaneously into the right femoral vein and then advanced until in contact with the right atrium. It was connected to an Elema-Schönander Model EM 145 external pacemaker unit. The stability of pacing was checked 10 - 15 beats/min above resting heart rate from a continuous ECG record. Voltages less than 3 V were used.

Heart rate was increased in steps of 10 beats/min, the first two or three steps lasting one minute each and subsequent steps lasting two minutes each. If only slight angina were experienced at two minutes, heart rate

was increased by a further 5 beats/min until the patient had moderate angina. Sampling from artery and coronary sinus was begun when two minutes had elapsed at that heart rate at which moderate angina developed. Pacing was continued at the same heart rate until sampling was complete.

A second pacing 90 minutes later, in the presence of an infusion of nicotinic acid, was carried out in precisely the same way as the first pacing in that subject. First and second sampling times did not differ by more than 15 seconds and samplings were usually completed within 3 minutes.

Electrocardiographs were recorded from leads CR 5 and CR 7 (2). A synchronous record of blood pressure was made from an intra-arterial catheter throughout pacing, except at the time of sampling because the same arterial catheter was used.

Treatment of Samples

Blood for paper electrophoresis of lipoproteins (47) was allowed to clot in a glass test tube.

Paired samples of arterial and coronary sinus blood were drawn into heparinised glass syringes for the estimation of oxygen and pH. All other blood samples were drawn into unheparinised plastic syringes.

Samples for the estimation of blood lactate and pyruvate were deproteinised within 30 seconds with perchloric acid.

Samples for all other determinations were immediately transferred to heparinised tubes and placed in iced water.

Aliquots for the estimation of blood glucose were taken from paired arterial and coronary sinus samples and deproteinised with perchloric acid within 5

minutes of the blood being drawn.

Where nephelometric estimation of exogenous triglyceride was required, heparinised blood from artery or coronary sinus was pooled and thoroughly mixed. It was centrifuged twice at +4°C or at room temperature for 10 minutes at 60 x g to obtain plasma (13, 14).

Blood for triglyceride, FFA, ^{3}H-FFA, ^{3}H-triglyceride, glycerol or hormone determinations was centrifuged at +4°C for 15 minutes at 1500 x g.

Plasma for glycerol or hormone determinations was frozen at -20°C.

Analytical Methods

Oxygen saturation of blood was measured spectrophotometrically (38). Oxygen tension was measured with a polarographic electrode (Instrumentation Lab. mod. 113). Oxygen content of blood was calculated from oxygen saturation, haemoglobin concentration and oxygen tension (38). Blood pH values were determined with Micro-Astrup equipment.

Plasma triglyceride concentration was determined by measuring total plasma glyceride-glycerol by an Auto Analyzer technique (40). The values were corrected for free glycerol which is measured together with glyceride-glycerol by this method (7).

"Exogenous plasma triglyceride" concentration was measured by a nephelometric technique using Intralipid^{R}-S fat emulsion, from the same flask as that from which infused emulsion came, as standard and the fasting plasma as blank (13, 14). To a small extent, endogenous plasma triglyceride contributed to the light scattering intensity (LSI) of "exogenous plasma triglyceride" and this contribution will have increased as the infusions proceeded (8, 67). However, unless endogenous triglyceride concentration changes across the coronary circulation, in the presence

of exogenous triglyceride, this will not affect the assessment of arterial-coronary sinus differences in concentration (13). An assumption which is made in using the technique to measure arterio-venous differences is that the spectrum of particle size does not change between artery and vein. If it did, LSI could alter without a change in concentration (13, 67). However, we have previously provided evidence, by making chemical and nephelometric measurements and by examining arterial and coronary sinus samples electron-microscopically, that changes in particle size spectrum are unlikely to account for changes in LSI across the coronary circulation (13).

Plasma glycerol was measured by an enzymatic fluorometric method (20).

Total plasma cholesterol was determined by an Auto Analyzer technique (69).

Plasma FFA were measured by the method of Trout et al (70).

Blood glucose (36), lactate (48) and pyruvate (9) concentrations were measured with enzymatic methods.

Plasma insulin was determined using an insulin immunoassay kit (Radiochemical Centre, Amersham, England) based on the double antibody method described by Hales and Randle (33). Plasma growth hormone was determined using the double antibody radioimmunoassay of Cerasi et al (16). Plasma glucocorticoid was measured using the fluorometric method of De Moor et al (23) as modified by S. Laurell (personal communication). The method measures total unconjugated plasma cortisol and corticosterone.

Whole body oxygen uptake and respiratory quotient were measured before and during the infusions of glucose and Intralipid^{R}-S (Paper V). Expired air was collected in a Douglas bag and analysed for oxygen and carbon dioxide

by the Haldane technique. The oxygen uptake and RQ values were then calculated.

Radioisotope Determinations

Plasma lipids were extracted according to the method of Boberg (6, 43). FFA and triglyceride fractions were separated by thin-layer chromatography and the radioactivity counted in a Packard liquid scintillation spectrometer. Corrections for quenching were carried out with internal standards (43). The radioactivity in the ^{3}H-palmitate infusate was determined from samples taken from the infusion syringe at the end of the study. These were added to aliquots of plasma, extracted, separated by thin-layer chromatography and the radioactivity in the FFA spot measured.

Plasma ^{125}I-albumin radioactivity was determined according to Wetterfors et al (73).

Calculations

The myocardial extraction of a substrate was defined as the difference in concentration between arterial and coronary sinus blood (Ca-cs). The myocardial extraction of plasma FFA measured radioisotopically was obtained by dividing the arterial-coronary sinus difference in radioactivity by the arterial specific radioactivity. Release of FFA into the coronary circulation was taken as the difference between FFA extraction measured radioisotopically and chemically.

The plasma FFA turnover rate (μmol/min) was calculated by dividing the product of the infusate radioactivity (cpm/ml) and infusion rate (ml/min) by the arterial FFA specific radioactivity (cpm/μmol) (75).

The relative contribution of different blood substrates, if completely oxidised, to myocardial oxidative metabolism was estimated by calculating

the oxygen extraction ratios (OER's) according to the following formula:

$$\text{OER \%} = \frac{\text{Ca-cs S} \cdot \text{EqO}_2}{\text{Ca-cs O}_2} \times 100$$

where Ca-cs S = myocardial extraction of substrate S (μmol/l blood) (it is necessary to convert concentrations in plasma to those in whole blood, using haematocrit)

Ca-cs O_2 = myocardial extraction of oxygen (μmol/l blood)

EqO_2 = oxygen equivalent for substrate S (triglyceride fatty acid, 73.5; FFA, 24.5; glucose, 6; lactate, 3; pyruvate, 2.5)

Results and Discussion

Free Fatty Acids and Myocardial Carbohydrate Metabolism (Papers I, II, V and VI)

(a) In Fasting Healthy Men

(i) Theoretical Considerations

Myocardial extraction of FFA was found to be significantly and positively related to arterial plasma FFA concentration in fasting subjects some of whom had nicotinic acid infusions (Table I). The correlation coefficient was high and may not have been improved by relating myocardial uptake of FFA (extraction x coronary blood flow) to arterial plasma concentration. The relationship between extraction and concentration amounts to the same as that between uptake (extraction x flow) and plasma substrate flow (concentration x flow). It thus appears that, in the fasting state, plasma FFA flow (or concentration) is a suitable predictor of myocardial FFA uptake (or extraction).

Table 1 Relationships between myocardial extraction and concentration in arterial blood of various substrates in healthy resting subjects.

		FASTING WITH AND WITHOUT NICOTINATE r	FASTING AND PAREN-TERALLY-FED r
Ca-cs FFA	Ca FFA	0.86^{xxx} (25)	0.24^{ns} (37)
Ca-cs GLUCOSE	Ca GLUCOSE	0.59^{xx} (25)	0.39^{x} (37)
Ca-cs LACTATE	Ca LACTATE	0.66^{xxx} (25)	0.55^{xx} (30)
Ca-cs PYRUVATE	Ca PYRUVATE	0.84^{xxx} (25)	0.74^{xxx} (30)

(1) Correlation coefficients (r) are shown

(2) Ca is the concentration in arterial blood of glucose, lactate or pyruvate or, in arterial plasma, of FFA. Ca-cs is the arterial - coronary sinus difference in concentration.

(3) The number of observations is indicated in parentheses beneath the corresponding r.

(4) Significance is indicated by ns ($P > 0.05$), x ($P < 0.05$). xx ($P < 0.01$) or xxx ($P < 0.001$).

It should be possible, also, to relate myocardial carbohydrate extraction to plasma FFA concentration as a way of examining relationships between myocardial carbohydrate and FFA metabolisms (Table 2).

If uptake (extraction x flow) should be related to arterial substrate concentration rather than to plasma substrate flow, the effect of flow can be eliminated, when interrelationships are under consideration, by relating the extraction of one substrate to that of another. In Paper I, the myocardial extractions of carbohydrate substrates have been related to the extraction of FFA.

The relation of the extraction of one substrate to that of another serves also to bring the relationship between substrates closer to cellular events. Although it cannot be said that all extracted substrate is oxidised immediately, in steady state conditions it is reasonable to assume that the degree of extraction of a substrate is related to the extent of its oxidation. An important assumption here is that intracellular events have reached steady-state conditions at the time that they have been reached in plasma - it is only the latter which it has been possible to assess in the present investigation. For substrates with a rapid turnover rate like FFA, this would appear to be a reasonable assumption. Most et al (51) found a fairly steady-state production of $^{14}CO_2$ by the heart after about 30 minutes of infusion of ^{14}C-labelled FFA. Therefore, in the studies referred to here, if two substrates, one of them FFA, had related extractions, their oxidations have been considered related.

In addition, where the concentration of one substrate, FFA in the present context, has been altered essentially independently of those of other substrates, changes in extraction of those other

TABLE 2 Relationships between myocardial extraction of various substrates and various arterial concentrations of substrates in healthy resting subjects.

FASTING, WITH AND WITHOUT NICOTINATE					FASTING AND PARENTERALLY-FED				
VARIABLES		r	ELIMINATING	$r_{12\cdot3}$	VARIABLES		r	ELIMINATING	$r_{12\cdot3}$
1	2		3		1	2		3	
Ca-cs GLUCOSE	Ca FFA	-0.66^{xxx} (25)	Ca GLUCOSE	-0.59^{xx}	Ca-cs FFA	Ca GLUCOSE	-0.69^{xxx} (37)	Ca FFA	-0.79^{xxx} (37)
Ca-cs LACTATE	Ca FFA	-0.72^{xxx} (25)	Ca LACTATE	-0.61^{xx}	Ca-cs FFA	Ca LACTATE	-0.60^{xxx} (30)	Ca FFA	-0.60^{xxx} (30)
Ca-cs PYRUVATE	Ca FFA	-0.56^{xx} (25)	Ca PYRUVATE	-0.47^{x}	Ca-cs FFA	Ca PYRUVATE	-0.46^{xx} (30)	Ca FFA	-0.58^{xx} (30)

(1) See footnotes to Table 1.

(2) $r_{12\cdot3}$ is the partial correlation coefficient.

substrates can be considered secondary to a change in concentration of the one substrate.

Since it is possible that FFA might affect myocardial carbohydrate extraction in a variety of ways, the contribution of other factors to the relationships must be considered. It is for this reason that partial correlation and multiple regression analyses have been done.

(ii) At Rest

When fasting plasma FFA concentrations were lowered with a constant infusion of nicotinic acid (11) myocardial extractions of glucose, lactate and pyruvate were increased (Table 2).

Similarly significant negative relationships were found when the myocardial extractions of carbohydrate substrates were related to the extraction of FFA as when extraction of carbohydrate was related to plasma concentration of FFA.

FFA have been shown to affect myocardial carbohydrate metabolism in man since their plasma concentrations,and only to a small extent the concentrations in blood of carbohydrates,were affected by nicotinic acid.

In Papers I and II, partial correlation analysis and multiple regression analysis have shown that myocardial glucose and lactate extractions can be affected by FFA extraction independent of the extractions of other carbohydrate substrates or of the hormones insulin, growth hormone or glucocorticoid. The two significant negative extraction relationships, glucose to FFA and lactate to FFA, can be explained if the oxidation of FFA leads to inhibition of pyruvate dehydrogenase (26, 72) (Figure 1).