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Substrate Competition in Human Myocardial Metabolism

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Introduction

The isolated perfused heart has for some time been the main source of information about competition among myocardial substrates [17–20, 26]. The findings that free fatty acids (FFA) and ketone bodies could decrease glucose uptake by isolated hearts were incorporated into 'the glucose-fatty acid cycle' hypothesis advanced by RANDLE *et al.* in 1963 [19]. It remained, however, to be shown, firstly, that the human heart *in vivo* handled its substrates in this way; secondly, what the relative importance of the interactions was, and thirdly, whether other substrates competed for extraction by the myocardium.

The application of coronary sinus catheterisation to this problem in man has provided data on substrate competition [2, 13, 22–25]. It has also allowed what is now thought to be a fairly complete description of substrates found in blood and used in myocardial energy metabolism [4, 7, 11, 12, 25]. The carbohydrate substrates are glucose [1, 11], lactate [1, 11] and pyruvate [1, 11] and the lipid substrates FFA [8, 11], ketone bodies [2], and triglyceride (TG), both endogenous and exogenous [4, 25].

Methods

Subjects were male, either healthy [11, 14, 25] or patients with angina pectoris. In the latter group no patient had had a myocardial infarction less than 5 months before the study and none was known to be diabetic [10].

1 Deceased.

Studies were conducted in the supine position after an overnight fast. Simultaneous arterial and coronary sinus blood samples were taken from catheters at these sites [4, 11, 25]. A citrate drip was maintained through the coronary sinus catheter and no heparin was used [4, 11, 25]. Any constant infusions were made via arm or central veins. Infusions used were ^{3}H-palmitate bound to human albumin, sodium nicotinate (200 or 400 mg/h), glucose (0.31–0.32 g/min) and Intralipid®-S, a modified form of the fat emulsion in sorbitol rather than glycerol (0.16–0.17 g triglyceride/min) [4].

If subjects exercised, this was on a cycle ergometer at 50 % of the load which produced a heart rate of 170/min after 6 min of exercise (W_{170}) [11]. It was intended that subjects exercise for 2 h and all exercised for at least 65 min. In those suffering from angina pectoris, moderate angina was induced by atrial pacing. Samples of arterial and coronary sinus blood were taken after 3 min of angina and while the same heart rate was maintained. Each subject was paced in the same way a second time 90 min later, in some subjects in the presence of an infusion of nicotinic acid.

Blood samples were collected for glucose, lactate, pyruvate, FFA, total plasma triglyceride, exogenous triglyceride, glycerol, insulin, growth hormone, glucocorticoid and blood gas determinations as previously described [4, 11, 25].

The relative contribution of different blood substrates, if completely oxidised, to myocardial oxidative metabolism was estimated by calculating the oxygen extraction ratios (OER) according to the following formula:

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

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

Fasting State

Healthy Individuals

Significant negative correlations are to be found between the myocardial extractions of glucose, lactate or pyruvate and arterial plasma FFA concentrations in healthy resting men [13]. The additional finding that infusion of an antilipolytic or plasma FFA lowering agent, nicotinic acid, leads to increased myocardial carbohydrate extraction and OERs, both at rest and during prolonged exercise (fig. 1), suggests that plasma FFA are

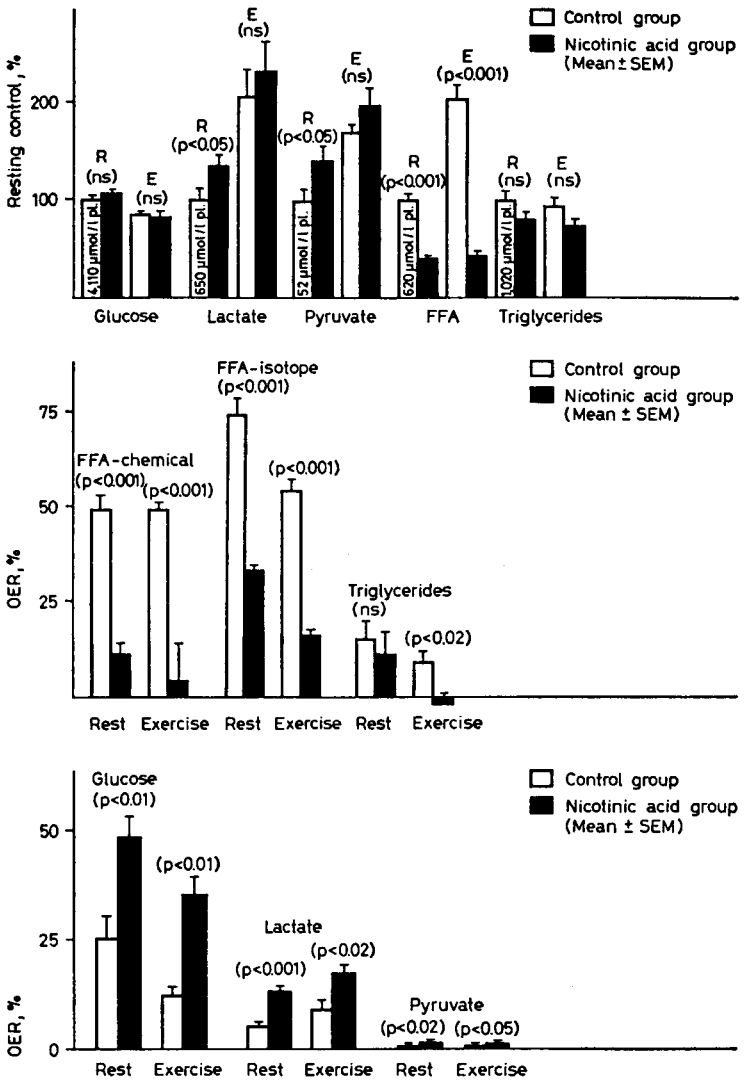

Fig. 1. Effect of nicotinic acid on myocardial metabolism in man at rest and during prolonged submaximal exercise. 25 subjects were studied, each at rest and during exercise; 10 of the 25 subjects received an infusion of nicotinic acid. *a* Changes in substrate concentrations. b Oxygen extraction ratios for lipid substrates. 'FFA chemical' is net change in FFA across the coronary circulation; 'FFA isotope' is taken to be actual extraction of FFA. *c* Oxygen extraction ratios for carbohydrate substrates.

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

		Fasting with and without nicotinate r	Fasting and parenterally-fed r
Ca-cs FFA	Ca FFA	0.86^{c} (25)	0.24^{ns} (37)
Ca-cs glucose	Ca glucose	0.59^{b} (25)	0.39^{a} (37)
Ca-cs lactate	Ca lactate	0.66^{c} (25)	0.55^{b} (30)
Ca-cs pyruvate	Ca pyruvate	0.84^{c} (25)	0.74^{c} (30)

Correlation cofficients (r) are shown.
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.
The number of observations is indicated in parentheses alongside the corresponding r.
Significance is indicated by ^{ns} ($p > 0.05$), ^{a} ($p < 0.05$), ^{b} ($p < 0.01$) or ^{c} ($p < 0.001$).

a determinant of myocardial carbohydrate metabolism. Moreover, myocardial extraction of FFA, closely related to arterial FFA concentration in these circumstances (table I), can also be negatively correlated with myocardial extraction of carbohydrate [23]. Since at least two carbohydrates, glucose and lactate, are involved when FFA extraction is altered by nicotinic acid, it is likely that FFA oxidation results, predominantly, in an inhibition of pyruvate dehydrogenase [13, 23, 24].

The factors which might affect the myocardial extraction of carbohydrate substrate are the substrate's own concentration in blood, the extraction of other carbohydrates or of lipids, hormones and cardiac work. Multiple regression analysis was used to find, from experimental data, the best equation to predict glucose extraction by the human heart in resting subjects [24]. The lipids considered were FFA, its presumed actual extraction measured isotopically and its release (actual minus net extraction), and endogenous plasma triglyceride. Hormones considered were insulin, growth hormone and glucocorticoid. Significant independent variables were actual FFA extraction (decreases Ca-cs glucose), arterial insulin immunoreactivity (increases Ca-cs glucose), and arterial gluco-

corticoid concentration (decreases Ca-cs glucose). For prolonged exercise, multiple regression analysis using the same independent variables failed to produce an equation better than that on simple linear regression,

$$\text{Ca-cs glucose} = 357 - 0.92^{b}\ \text{Ca-cs FFA isot}$$
$$[b\ (p < 0.01),\ \text{F-ratio} = 9.08\ (p < 0.01)].$$

The inclusion in the equation of heart rate as an independent variable related to cardiac work [11] did not provide a better regression equation.

In these studies, evidence of lipolysis of myocardial triglyceride was obtained [11, 14]. At rest, during infusions of ^{3}H-palmitic acid complexed to albumin, unlabelled free fatty acid appeared in the coronary sinus ('FFA release'), and, during exercise, glycerol appeared in the coronary sinus. Nicotinic acid has been shown *in vitro* to inhibit isoprenaline-stimulated myocardial lipolysis [6]. Apparently it does so incompletely or to one triglyceride pool and not another [21] since nicotinic acid treated rats actually have a reduced myocardial triglyceride content [3]. In subjects receiving nicotinic acid, at rest and exercising, evidence of FFA release was found. Considering myocardial respiratory quotients and OERs together, it seemed likely that, during exercise with nicotinic acid, myocardial triglyceride stores were being utilized [14].

Coronary Heart Disease

The patients with coronary heart disease (CHD) had higher arterial FFA concentrations and myocardial FFA extractions at rest than did healthy subjects. However, myocardial glucose extraction was not correspondingly decreased as might have been expected on the basis of glucose/FFA relationships in the healthy heart. In those with CHD, myocardial extraction of lactate was less and of pyruvate more for a given arterial concentration than it was for healthy subjects. One problem in assessing these differences is that the groups were not matched. The age ranges for the healthy subjects was 22–42 (mean 29) years and for the CHD group 52–67 (mean 58) years. Another problem with the ischaemic heart is that its metabolism is likely to be heterogenous so that changes in a small ischaemic area may be masked.

Atrial pacing to the point where those with coronary heart disease experienced angina pectoris (mean heart rate 127/min) produced no significant changes in the relative contribution of substrates to myocardial oxidative metabolism (fig. 2), although the combined OER of the measured

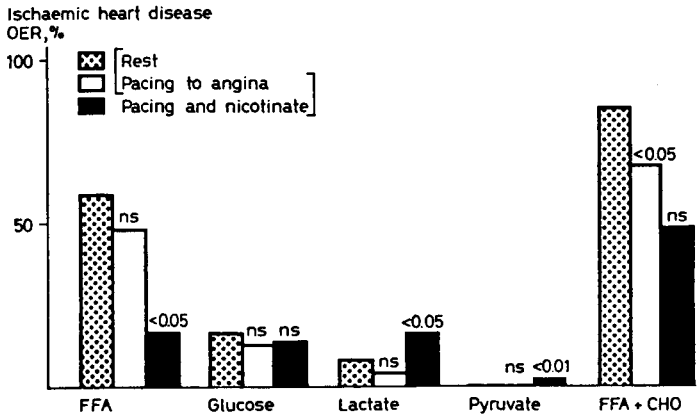

Fig. 2. Oxygen extraction ratios for myocardial substrates in patients suffering from angina pectoris. All subjects (9) were subject to atrial pacing twice; in 5 subjects the second pacing took place during a nicotinate infusion. Significances shown are for the comparison pacing with rest and for the comparison pacing with pacing during nicotinate infusion.

substrates (FFA, glucose, lactate and pyruvate) fell (fig. 2). Other workers have found that the ischaemic heart extracts more glucose when subjected to pacing stress [16]. Second atrial pacings in the presence of nicotinic acid resulted in significant falls in myocardial FFA extraction and OER and rises in myocardial lactate and pyruvate extractions and OERs (fig. 2); glucose extraction and OER were unaffected. That both lactate and pyruvate change in the same direction suggests that there was less PDH inhibition. Glycolysis or the hexose monophosphate shunt [9] could be proceeding at an increased rate without an increase in glucose extraction if glycogenolysis were increased. The myocardial respiratory quotient was 0.92 during atrial pacing with nicotinate and this indicates that the heart was using 74 % carbohydrate and 26 % lipid. The combined carbohydrate OER at this time of 33 % should be a complete description of blood-derived carbohydrate. This leaves 41 % of myocardial oxidative metabolism to be made up by myocardial glycogen stores. Thus, it appears likely that nicotinic acid, although not leading to an increased glucose extraction in angina pectoris, may lead to an increase in anaerobic metabolism. More direct information is required, however.

Fed State (Healthy Individuals)

Glucose Extraction

(1) Three subjects were given a fatty meal and 3 and 4 h later the myocardial extractions of various substrates were examined (table II). The glucose extraction when glucose and Intralipid®-S were infused (intravenous fed state, IVFS) was appreciably higher than during alimentary lipaemia (table II). When the fasting and parenteral feeding observations were considered together, there was a significant correlation between Ca-cs glucose and Ca glucose (table I). These findings suggest that Ca glucose can be a determinant of Ca-cs glucose.

(2) A significant negative relationship was found also between Ca-cs glucose and Ca FFA and this did not depend on Ca glucose ($r_{12.3}$, the partial correlation coefficient $= -0.52$, $p < 0.01$) or Ca exogenous triglyceride ($r_{12.3} = -0.43$, $p < 0.01$).

(3) A relationship between Ca-cs glucose and Ca exog. TG for the combined fasting and IVFS observations depended on Ca glucose ($r_{12.3} = 0.11$). Glucose extraction during alimentary lipaemia was lower than in fasting subjects, but not significantly so.

(4) An additional factor in the difference between Ca-cs glucose during alimentary lipaemia and parenteral feeding might be the higher Ca insulin in the latter group (table II).

FFA Extraction

(1) Despite the fact that Ca FFA was maintained at fasting concentrations during parenteral feeding with glucose and Intralipid®-S, myocardial FFA extraction was significantly less than in the fasting state (fig. 3).

This was not simply due to lipolysis of exogenous plasma triglyceride in the coronary circulation, since both actual (isotopic) and net (chemical) FFA extractions were affected. Furthermore, Ca-cs FFA was no longer significantly related to Ca FFA (table I).

For the combined fasting and parenteral feeding data, Ca-cs FFA was significantly and negatively correlated with Ca glucose ($r = -0.69$, $p < 0.001$), Ca lactate ($r = -0.60$, $p < 0.001$), and Ca pyruvate ($r = -0.46$, $p < 0.01$) and these relationships did not appear to depend on Ca FFA or Ca exogenous triglyceride. This is in contrast to studies with the isolated heart where it has not been possible to demonstrate an effect of glucose on FFA uptake [20].

Table II. Comparison of myocardial extractions of glucose, exogenous triglyceride and FFA in the fasting state, oral fed state and intravenous fed state

	Fasting	Alimentary lipaemia	IVFS
Ca glucose, μmol/l blood	4,110 ± 140 (15)	4,590 ± 140 (6)	8,410 ± 290 (18)
Ca-cs glucose	190 ± 40c (15)	80 ± 40ns (6)	290 ± 50c (18)
Ca exog. TG, μmol/l plasma	0	1,190 ± 160 (6)	2,580 ± 160 (38)
Ca-cs TG	0	70 ± 20a (6)	80 ± 20c (38)
Ca FFA, μmol/l plasma	620 ± 50 (15)	1,110 ± 50 (6)	710 ± 30 (38)
Ca-cs FFA	170 ± 20c (15)	130 ± 40a (6)	70 ± 10c (38)
Ca insulin, μU/ml	–	9.3 ± 1.7 (6)	26.8 ± 3.2 (36)

Mean ± SEM are shown.
Number of observations is shown in parentheses.
Significance of a myocardial extraction is indicated by ns ($p > 0.05$), a ($p < 0.05$), and c ($p < 0.001$).

(2) Another reason for the decreased FFA extraction during parenteral feeding might be that FFA and triglyceride fatty acid compete for extraction. However, the significant negative relationship between Ca-cs FFA and Ca exogenous triglyceride ($r = -0.54$, $p < 0.001$) was lost when Ca glucose ($r = -0.11$) or Ca lactate ($r = -0.23$) was taken into account in a partial correlation analysis. Nevertheless, because of the design of the experiment, a synergistic effect of carbohydrate and triglyceride on Ca-cs FFA cannot be excluded and more work is needed to clarify this point.

Although Ca FFA during alimentary lipaemia was almost double that found for fasting subjects, Ca-cs FFA was not significantly different (table II). This applied also to Ca-cs FFA examined isotopically. Since the essential difference between alimentary lipaemia and the fasting state was the presence in blood of exogenous triglyceride, it seems likely that this has interfered with the correlation which is found in the fasting state between Ca-cs FFA and Ca FFA.

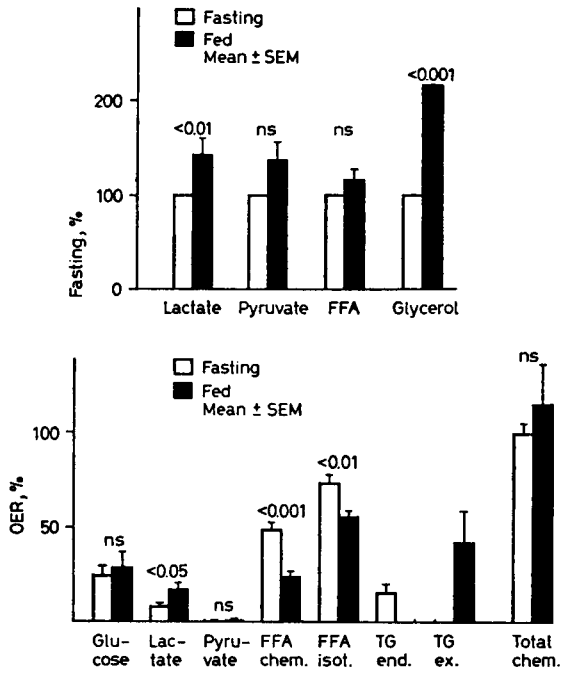

Fig. 3. Effect of parenteral glucose and fat emulsion (Intralipid®-S), administered simultaneously, on non-infused arterial substrate concentrations (upper figure) and on myocardial OERs (lower figure). FFA chem. = FFA OER measured chemically; FFA isot. = FFA OER measured radioisotopically; TG end. = endogenous plasma triglyceride; TG ex. = exogenous plasma triglyceride; total chem. = total OER calculated from chemically determined FFA extractions and either chemically or nephelometrically determined triglyceride extractions.

Endogenous Plasma Triglyceride Extraction

During these studies, endogenous plasma triglyceride was labelled with ^{3}H-palmitic acid. This allowed the assessment of its extraction independent of exogenous plasma triglyceride. Whereas a significant myocardial extraction of endogenous plasma triglyceride was found in this way for the fasting group, none could be found in the parenterally fed group (fig. 3). This raises the possibility that endogenous and exogenous plasma triglycerides compete for extraction by the heart. It is also possible, however, that the infusion of glucose in conjunction with that of fat emulsion has been responsible for the depressed endogenous plasma triglyceride extraction.

Relation of Oxygen to Substrate Metabolism

Under fasting conditions healthy subjects showed a significant positive correlation between myocardial oxygen extraction and myocardial FFA extraction [23]. For all observations made at rest, including those with nicotinate, the correlation coefficient (r) was 0.45 ($n = 57$, $p < 0.001$); without nicotinate r was 0.53 ($n = 47$, $p < 0.001$). Those observations made during prolonged exercise without nicotinate had an r of 0.55 ($n = 15$, $p < 0.05$). These relationships did not depend on mutual correlations with heart rate at rest, but the relationship during prolonged submaximal exercise did depend on heart rate. Carbohydrate extraction was not significantly correlated with oxygen extraction. These findings raise the possibility that, at rest, when the human heart used FFA as a substrate, its oxygen consumption is greater than when it uses carbohydrate. Evidence has been obtained for the isolated rat heart [5] and for the intact dog heart [15] that this is so. During prolonged exercise, however, this relationship is either lost or obscured by other factors affecting myocardial oxygen consumption.

Summary

The extractions of various substrates by the human heart have been measured to assess competition between substrates. In fasting healthy men an alteration in plasma free fatty acids (FFA) or their extraction led to a reciprocal change in myocardial carbohydrate metabolism such that pyruvate dehydrogenase seemed the enzyme chiefly involved. When angina pectoris was induced by atrial pacing in persons suffering from this disorder, a reduction in FFA extraction was associated with increases in lactate and pyruvate extractions but not in glucose extraction; nevertheless, glycolysis may have been more active. Reduced FFA extraction during parenteral feeding with glucose and a fat emulsion may have been due to changes in carbohydrate substrates; during alimentary lipaemia exogenous plasma triglyceride seemed responsible for decreased fractional extraction of FFA by the heart. Evidence suggesting competition between endogenous and exogenous plasma triglyceride was also obtained. In healthy fasting individuals FFA may stimulate oxygen metabolism.

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