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# The Role of Fatty Acid and of Hormones in the Determination of Myocardial Carbohydrate Metabolism in Healthy Fasting Men

M. L. Wahlqvist, L. Kaijser, B. W. Lassers, H. Löw, and L. A. Carlson

Department of Geriatrics, Uppsala University, Uppsala, King Gustaf V Research Institute, Stockholm and Departments of Clinical Physiology and of Endocrinology, Karolinska Hospital, Stockholm, Sweden

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*Abstract.* In earlier studies myocardial extraction (MEx) of lipid and carbohydrate substrates was measured in 25 healthy, fasting men under different conditions (rest, exercise, sodium nicotinate infusion). It was found that, at rest, MEx of glucose, lactate, pyruvate and FFA were positively correlated to their respective arterial concentrations. During exercisesuch correlations were found for lactate, pyruvate and FFA. Furthermore MEx, at rest and at exercise, of glucose, lactate and pyruvate were negatively correlated to arterial FFA levels or to MEx of FFA. In the present investigation the role of various hormones in regulating MEx of carbohydrate substrates was analysed by determination of arterial concentrations of hormones in the earlier studies. MEx of glucose was not significantly correlated to arterial concentrations of either insulin, growth hormone or glucocorticoid. MEx of the other two carbohydrate substrates showed weak correlations to growth hormone levels. Furthermore, since the interrelationships which exist *in vivo* between MEx of a substrate and (1) arterial concentration of the substrate, (2) hormones and (3) extraction of other substrates are complex, simple correlation analysis between these variables may be inadequate. For this reason the relationship of MEx of carbohydrate substrates to the above mentioned factors was examined by multiple regression analysis in order to discover to which factors the extraction was significantly and independently related. This analysis showed that MEx of glucose at rest was negatively related to MEx of FFA and to concentration of glucocorticoid, but positively to insulin levels and not related to arterial glucose concentration. The multiple regression equations suggested that MEx of FFA might affect MEx of carbohydrate substrates in part through inhibition of pyruvate dehydrogenase. Multiple regression analysis has thus permitted the demonstration of the relevance of certain control mechanisms postulated from studies with the isolated perfused heart to the situation *in vivo* where complex interrelationships often invalidate simple correlations. For example, the dependence of MEx of glucose on FFA extraction, insulin and glucocorticoid is quite consistent with the effects of these factors, one by one, observed *in vitro*. The multiple regression equation also gave quantitative estimates. Thus for a 10 per cent increase in either FFA extraction, insulin or glucocorticoid concentrations the equation indicated that such a change in one of the independent variables would alter MEx of glucose by —17%, +24% or —13% respectively.

*Key words:* Free fatty acids, glucose, lactate, pyruvate, substrate metabolism, insulin, growth hormone, corticosteroids, multiple regression analysis, carbohydrate uptake, human heart.

By 1940 it was recognized that glucose [40], lactate [28] and pyruvate [7] could be utilized as sources of energy by the mammalian heart, and in the early 1950's this was established for the human heart as well [2, 22, 23]. Several factors could determine the extent to which each of these substrates contributes to myocardial metabolism. There is evidence that their own concentrations in arterial blood are important [21, 24, 25]. In 1922, shortly after its discovery, insulin was found to increase glucose uptake by the isolated mammalian heart [27]; this has been confirmed *in vitro* [4, 43, 45, 65] and *in vivo* [23, 25]. Epinephrine, according to some reports [63, 66], and glucagon *in vitro* [34] but not *in vivo* [6], can also increase myocardial glucose utilization. Other hormones, growth hormone [45, 47, 50, 54] and glucocorticoid [45, 47, 50, 54] can decrease myocardial glucose utilization. There has been no attempt, however, to define the role of hormones *in vivo* without either ablation of an endocrine gland or hormone administration.

Early indications that non-carbohydrate substrates might affect myocardial carbohydrate metabolism came from studies which demonstrated an increase in cardiac glycogen when blood ketone body concentration increased [35, 41]. These observations were supported by the findings (a) that fasting diabetics had low myocardial glucose, lactate and pyruvate extractions and also myocardial respiratory quotients consistent with fat oxidation [25] and (b) that acetoacetate rendered the perfused rat heart less sensitive to insulin [64]. In 1961, Shipp *et al.* [55] reported that free fatty acid (FFA) suppressed glucose uptake and utilization in the isolated rat heart although FFA uptake was not affected by glucose. Similar findings were reported by Newsholme *et al.* in 1962 [49] when they provided evidence that ketone bodies and FFA inhibited the phosphofructokinase reaction. Subsequently, Randle and coworkers [51] proposed "the glucose-fatty acid cycle", one aspect of which was that FFA affected the utilization of glucose by muscle. Several studies have confirmed the effect of FFA on glucose uptake in the isolated perfused heart [14, 29, 50, 52, 56] and others have shown that FFA can effect lactate uptake by the dog heart *in vivo* [30] and pyruvate uptake by the isolated perfused heart [19, 20] in a similar way. Bing *et al.* [3] found a negative correlation between myocardial carbohydrate and ketone extractions in man. More recently, sodium nicotinate administration, which lowers plasma FFA concen-

tration [9, 11] and may improve glucose tolerance [12, 48], has been shown to decrease myocardial FFA extraction and increase myocardial glucose, lactate and pyruvate extractions in man [38, 60].

The present study is an attempt to define more clearly the roles of hormones and of FFA in human myocardial carbohydrate metabolism both at rest and during increased cardiac work associated with exercise. Arterial concentrations of plasma insulin, growth hormone and glucocorticoid and also arterial-coronary sinus differences in concentration of various myocardial energy substrates have been measured in healthy subjects at rest and during prolonged exercise, both in the absence and presence of an intravenous infusion of sodium nicotinate.

## Methods

### *Design of Studies*

Twenty-five healthy male volunteers, aged between 21 and 42 years, were studied without premedication after an overnight fast. Detailed methodology and results on lipid and carbohydrate substrates have been published elsewhere [10, 32, 36, 37]. Teflon catheters were introduced into both a brachial artery and the coronary sinus for simultaneous blood sampling. Heparin was not administered. The study was started by an intravenous infusion of albumin-bound $^3$H-palmitate [36]. After 60 minutes blood samples were drawn. The the subjects exercised for between 65 and 125 minutes and blood samples were again taken. Exercise was performed in the supine position on a cycle ergometer at a constant work load. The work load was 50% of that which produced a heart rate of 170/minute after 6 minutes of exercise ($W_{170}$) during a preliminary exercise test [32, 57, 61]. It was intended that exercise should last for 120 minutes, a duration tolerated by most healthy subjects in the fed state at the load used [1]. However, since in the present investigation the subjects had fasted and some of them received sodium nicotinate, 15 out of 25 stopped earlier than 120 minutes, in most cases because of leg fatigue and, in some cases, where sodium nicotinate was infused, because subjects felt faint. The exercise sampling was made during the last 5 minutes of work in all subjects. In 10 of the 25 subjects, a constant intravenous infusion of sodium nicotinate (200 mg/hour in 3 and 400 mg/hour in 7 subjects) was maintained throughout, after a priming dose of 200 mg 5% sodium nicotinate intravenously.

### *Treatment of Samples and Analytical Methods*

Blood samples were drawn into unheparinised plastic syringes. Samples for determination of lactate and pyruvate were deproteinised immediately with perchloric acid. The absence of chylomicra, confirmation of the fasting state, was checked by paper electrophoresis of lipoproteins [39] from a sample of blood placed in an unheparinised tube. The remaining blood was transferred to heparinised test tubes, and aliquots for glucose determination immediately deproteinised with perchloric acid. The heparinised whole blood was kept for 30 to 60 minutes in iced water and then centrifuged at 4° C. The plasma so obtained was either extracted immediately for the determination of FFA and triglyceride (TG) concentrations and of FFA radioactivity or frozen at —20° C for subsequent hormone determination.

Blood glucose was assayed in duplicate on each of 10 aliquots of whole blood from each sample using a commercially available (AB Kabi, Stockholm) glucose oxidase method, based on that of Hjelm [31]. Blood lactate and pyruvate were assayed in duplicate by the enzymatic methods of Lundholm *et al.* [42] and Bücher *et al.* [8] respectively. Plasma FFA were assayed in quadruplicate according to Trout *et al.* [59]; the heptane phase was washed twice with 0.05% $H_2SO_4$. Plasma TG were assayed in triplicate on each of 10 extracts of each plasma sample by an Auto Analyzer technique [33] as described elsewhere [10]. Radioactivity in the plasma FFA was determined according to the method of Boberg [5]. Plasma insulin was determined in duplicate using an insulin immunoassay kit (Radiochemical Centre, Amersham, England) based on the double antibody method described by Hales and Randle [26]. Plasma growth hormone was determined in duplicate using the double antibody radioimmunoassay of Cerasi *et al.* [13]. Plasma glucocorticoid concentration was measured in duplicate using the fluorometric method of De Moor *et al.* [16] as modified by S. Laurell (personal communication). The use of chloroform instead of methylene chloride to extract glucocorticoid from the glass fibre papers (used as a supporting medium for plasma in the Laurell modification) resulted in lower blank values. The method measures total unconjugated plasma cortisol and corticosterone.

### *Calculations and Statistical Analysis*

The release of FFA into the coronary circulation was taken as the difference between FFA extraction measured isotopically and chemically [36]. Data from studies in the absence and presence of nicotinate infusion have been combined as either resting or exercise categories.

Test of significance of difference in concentration and linear regression analysis have been made according to Snedecor [58]. Linear multiple regression analyses have been carried out using the General Electric computer time-sharing service [21] according to Davies [15] and Draper and Smith [18].

## Results

### *At Rest*

*1. Substrate Extraction and Arterial Concentration* (Fig. 1). The myocardial extractions of glucose, lactate,

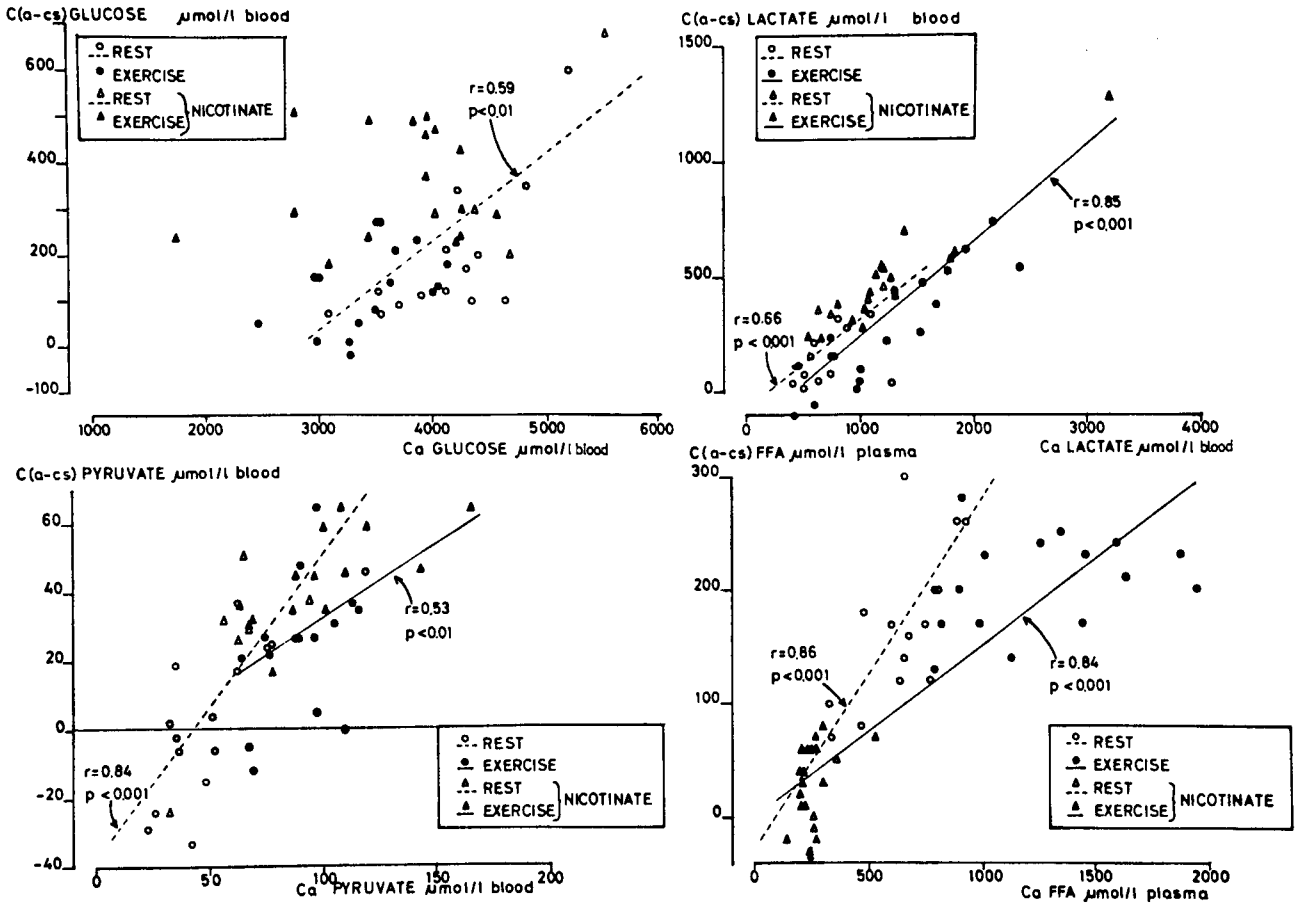


Fig. 1. Relationship between myocardial extraction and arterial concentration of glucose, lactate, pyruvate or FFA in 15 subjects not receiving sodium nicotinate and 10 subjects receiving an intravenous infusion of sodium nicotinate. Myocardial FFA extraction shown was measured chemically. Regression lines have been calculated for either the category "rest" or the category "exercise" combining non-nicotinate and nicotinate data

Table 1. Concentrations in arterial plasma of hormones in the four experimental categories

| | Rest | | Exercise | |
|---|---|---|---|---|
| | Without nicotinate | Nicotinate infusion | Without nicotinate | Nicotinate infusion |
| *Concentrations* | | | | |
| Insulin (µUnits/ml) | 17.9±0.9 (12) | 15.2±1.4$^{ns}$ (9) | 13.8±0.8$_b$ (12) | 11.4±1.4$^{ns}_c$ (9) |
| Growth hormone (ng/ml) | 10.4±1.2 (11) | 14.7±3.2$^{ns}$ (9) | 12.9±1.2$_{ns}$ (11) | 48.2±7.0$^a_c$ (9) |
| Glucocorticoid (µg/100 ml) | 12.5±1.9 (9) | 13.4±2.7$^{ns}$ (9) | 11.6±2.1$_{ns}$ (9) | 17.7±2.9$^{ns}_{ns}$ (9) |

(1) In each category, Mean±SEM and in parenthesis number of observations are shown.
(2) Significance of difference in concentration between the categories with and without nicotinate infusion, assesed by a *t*-test of the difference between two means is indicated by the superscripts ns ($P>0.05$) or $^a$ ($P<0.001$).
Significance of difference in concentration between the categories rest and exercise has also been assessed by a *t*-test of paired differences, and significance is indicated by the subscripts ns ($P>0.05$), b ($P<0.01$) or c ($P<0.001$).

pyruvate and FFA were significantly and positively related to their respective arterial concentrations.

*2. Carbohydrate Extraction and FFA Concentration and Extraction.* The myocardial extractions of glucose ($r=-0.66$, $n=25$, $p<0.001$), lactate ($r=-0.72$, $n=25$, $p<0.001$) and pyruvate ($r=-0.56$, $n=25$, $p<0.01$) were significantly and negatively related to arterial plasma FFA concentration.

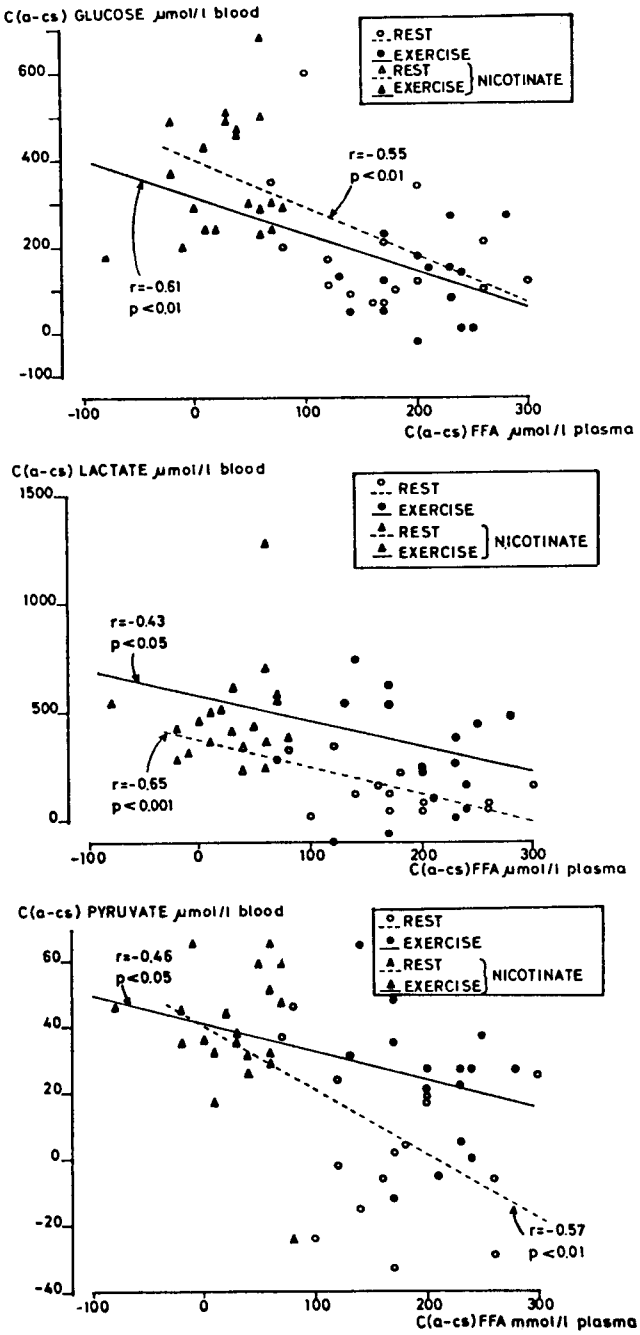


Fig. 2. Relationship between myocardial extraction of glucose, lactate or pyruvate and myocardial extraction of FFA measured chemically

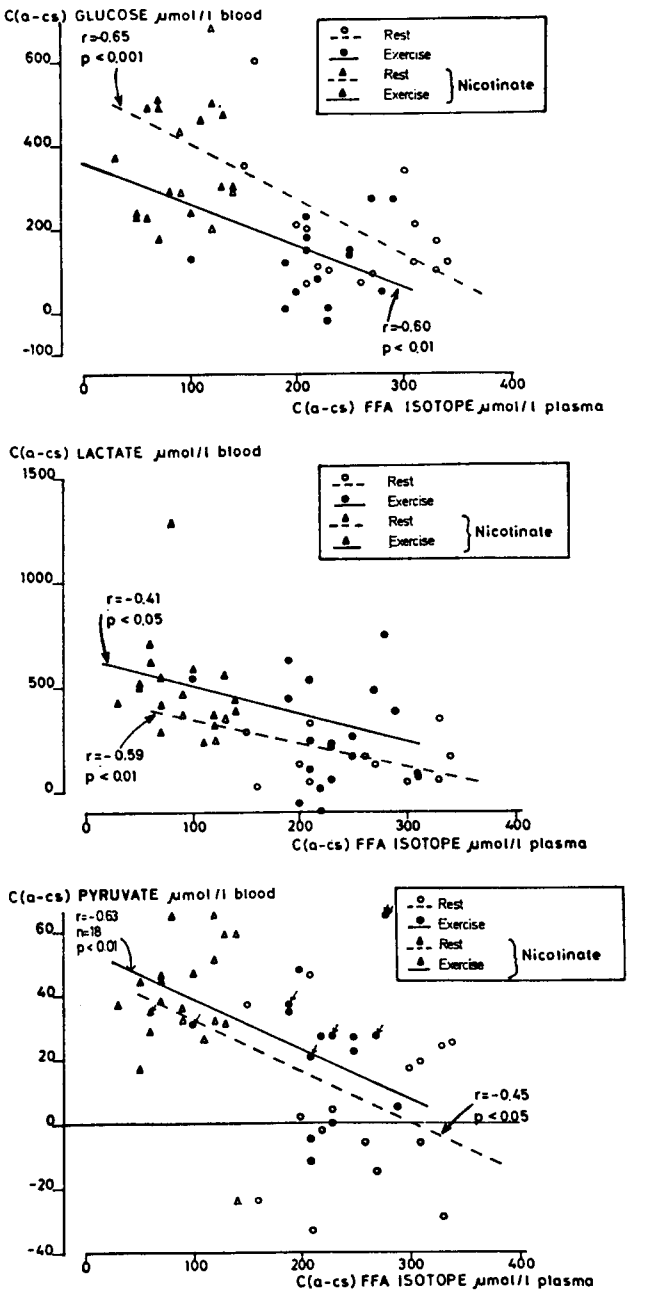


Fig. 3. Relationship between myocardial extraction of glucose, lactate or pyruvate and myocardial extraction of FFA measured radioisotopically. In the case of pyruvate extraction during prolonged exercise, the regression line shown is that for the 18 observations considered in the multiple regression analysis (Table 3). The 7 observations not included in the regression analysis are each marked with an arrow and the one of these 7 well-removed from the rest with a double-arrow (see text)

Significant negative relationships were found between the myocardial extractions of glucose, lactate and pyruvate and that of FFA whether FFA extraction was measured chemically or radioisotopically (Figs. 2 and 3).

*3. Carbohydrate Extraction and Arterial Hormone Concentration.* The arterial concentration of insulin, growth hormone or glucocorticoid did not differ significantly between the group of subjects that received sodium nicotinate and the group that did not (Table 1).

Table 2. Relationships between the myocardial extractions of carbohydrate and arterial plasma hormone concentrations

| | Linear correlation coefficients ($r$) | | |
|---|---|---|---|
| | Insulin ($n=21$) | Growth hormone ($n=20$) | Glucocorticoid ($n=18$) |
| Ca-cs Glucose | | | |
| Rest | $-0.34^{ns}$ | $0.12^{ns}$ | $0.10^{ns}$ |
| Exercise | $-0.11^{ns}$ | $0.36^{ns}$ | $0.37^{ns}$ |
| Ca-cs Lactate | | | $0.25^{ns}$ |
| Rest | $-0.08^{ns}$ | $0.45^{a}$ | $-0.25^{ns}$ |
| Exercise | $-0.33^{ns}$ | $0.53^{a}$ | $0.22^{ns}$ |
| Ca-cs Pyruvate | | | |
| Rest | $-0.10^{ns}$ | $0.54^{a}$ | $0.39^{ns}$ |
| Exercise | $-0.47^{a}$ | $0.38^{ns}$ | $0.31^{ns}$ |

(1) Observations at rest or during prolonged exercise in the absence and presence of nicotinate have been considered together.
(2) Significance of r is indicated by ns ($P>0.05$) or a ($P<0.05$).

Glucose extraction by the myocardium could not be significantly related to arterial insulin, growth hormone or glucocorticoid concentration (Table 2).

Lactate and pyruvate extractions were positively related at the 5% level to arterial growth hormone concentration (Table 2).

*4. Multiple Regression Analysis* (Table 3). Equations for the myocardial extractions of glucose, lactate and pyruvate have been written on the basis of multiple regression analysis. For a "complete" equation, 9 variables, including the substrate's own arterial concentration, the extractions of other myocardial substrates and plasma hormone concentrations, have been considered. Variables have then been removed successively and the F ratio[1] and partial regression coefficients (with their t-values) inspected to determine the equation most predictive of the extraction of the substrate in question (the "best" equation).

Myocardial glucose extraction was best predicted from the complete equation. The regression coefficients for FFA extraction measured radioisotopically, arterial insulin concentration and arterial glucocorticoid concentration were significant. Assuming that only one independent variable is altered at a time, a 10% increase in the arterial concentration of insulin or glucocorticoid would result, respectively in a 24% increase or a 13% decrease in myocardial glucose extraction. A 10% increase in FFA extraction would result in a 17% decrease in glucose extraction.

For lactate extraction, arterial lactate concentration and FFA extraction measured radioisotopically emerged with significant regression coefficients in the best multiple regression equation. At higher lactate concentrations and lower FFA extractions, more lactate would be extracted.

Increases in arterial pyruvate concentration, myocardial glucose extraction, arterial growth hormone concentration or glucocorticoid concentration would increase, and an increase in arterial insulin would decrease, myocardial pyruvate extraction.

*During Prolonged Exercise*

*1. Substrate Extraction and Arterial Concentration* (Fig. 1). The myocardial extractions of lactate, pyruvate and FFA were significantly and positively related to their arterial concentrations during prolonged exercise.

*2. Carbohydrate Extraction and FFA Concentration and Extraction.* The myocardial extractions of glucose ($r=-0.62$, $n=25$, $p<0.001$), lactate ($r=-0.48$, $n=25$, $p<0.05$) and pyruvate ($r=-0.54$, $n=25$, $p<0.01$) were significantly and negatively related to arterial plasma FFA concentration.

Significant negative relationships were found between carbohydrate extractions and FFA extraction (Figs. 2 and 3). It was only possible to measure all variables required in the multiple regression analysis for 18 of the total 25 observations made. Although the relationship between pyruvate and FFA extraction measured radioisotopically was significant for the 18 observations considered in the multiple regression equation (see below), it was not for the 25 observations shown in Fig. 3. This appeared to depend on one observation well removed from the regression line (marked in the figure) since the remaining 24 observations had an $r$ of $-0.54$ ($p<0.01$).

*3. Carbohydrate Extraction and Arterial Hormone Concentration.* Prolonged exercise led to a significant fall in arterial insulin concentration in both the group which received sodium nicotinate and that which did not (Table 1). It also led to a significant rise in arterial growth hormone concentration in the group which received sodium nicotinate (Table 1). It led to no significant change in plasma glucocorticoid concentration (Table 1).

Glucose extraction could not be significantly related to arterial insulin, growth hormone or glucocorticoid concentration (Table 2).

Lactate extraction was positively related at the 5% level to arterial growth hormone concentration (Table 2).

Pyruvate extraction was negatively related at the 5% level to arterial insulin concentration (Table 2).

*4. Multiple Regression Analysis* (Table 3). The best equation for the prediction of myocardial glucose extraction during prolonged exercise had a non-significant F-ratio. The only regression coefficient which was significant was that for triglyceride extraction. Its negative sign indicates that an increased myocardial

1 The F ratio was calculated from an analysis of variance tables for multiple regression [21] by dividing MSR (regression mean square) by MSE (error mean square). Its significance was read from variance-ratio (F) tables entering at $f_1$ and $f_2$. "$f_1$", the number of degrees of freedom for MSR, was the number of independent variables considered ($k$). "$f_2$", the number of degrees of freedom for MSE, was '$n$-$k$-1' where $n$ was the number of observations. In all cases $n$ was 18.

Table 3. Myocardial extractions of carbo-

| Dependent variable (y) ($C_{a-cs}$) | Selected equation | Independent variables (X) – partial regression coefficients (B) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Bo | Ca Glucose | Ca Lactate | Ca Pyruvate | $C_{a-cs}$ FFA Isot. | $C_{a-cs}$ TG |
| *Rest* | | | | | | | |
| Glucose | Complete and Best | 161 | $0.11^{ns}$ | — | — | $-2.09^{b}$ | $-0.47^{ns}$ |
| Lactate | Complete | −68 | — | $0.44^{c}$ | — | $-1.23^{ns}$ | $-0.72^{ns}$ |
| | Best | 51 | — | $0.45^{c}$ | — | $-0.64^{a}$ | — |
| Pyruvate | Complete and Best | −32 | — | — | $0.60^{c}$ | $0.08^{ns}$ | $-0.10^{ns}$ |
| *Exercise* | | | | | | | |
| Glucose | Complete | −25 | $0.08^{ns}$ | — | — | $-0.43^{ns}$ | $-4.29^{ns}$ |
| | Best | −43 | 0.07 | — | — | $-0.39^{ns}$ | $-4.22^{a}$ |
| Lactate | Complete | −397 | — | $0.43^{c}$ | — | $-0.35^{ns}$ | $0.54^{ns}$ |
| | Best | −336 | — | $0.43^{c}$ | — | — | — |
| Pyruvate | Complete | 31 | — | — | $0.21^{ns}$ | $-0.10^{ns}$ | $-0.19^{ns}$ |
| | Best | 55 | — | — | — | $-0.16^{b}$ | — |

(1) The multiple regression equations are of the form $y=Bo+B_1X_1+B_2X_2+B_3X_3\ldots$
(2) Abbreviations: FFA=free fatty acid; Isot.=measured radioisotopically; TG=triglyceride; $C_a$=arterial concentration; $C_{a-cs}$=myocardial extraction.
(3) Units used are μmol/l except for insulin (μUnits/ml), growth hormone (ng/ml) and glucorticoid (μg/100 ml). Carbohydrate substrates in blood, lipids and hormones in plasma.
(4) In all analyses $n=18$.

extraction of triglyceride might reduce myocardial extraction of glucose.

For lactate extraction during prolonged exercise, an equation of high predictive value was found. It included two independent variables, arterial lactate and growth hormone concentrations, both with highly significant regression coefficients.

Pyruvate extraction during prolonged exercise was best predicted from an equation containing only FFA extraction measured radioisotopically.

## Discussion

### *Rest*

*Myocardial Glucose Extraction.* At rest, myocardial glucose extraction was positively correlated with its concentration in arterial blood. However, in the multiple regression equation, arterial concentration did not have significant predictive value. This presumably means that the correlation is in fact determined by factors other than arterial glucose concentration itself, which have been included in the equation.

Myocardial glucose extraction was significantly and negatively related to FFA extraction on both direct linear correlation and multiple regression analysis. FFA extraction measured radioisotopically was, in turn, closely related to plasma FFA concentration ($r=0.90$, $p<0.001$). These findings support the "glucose-fatty acid cycle" hypothesis advanced by Randle and coworkers [51].

No significant direct linear correlations were found between myocardial glucose extraction and arterial plasma hormone concentrations. Yet multiple regression analysis revealed significant relationships for insulin and glucocorticoid in agreement with described effects of these hormones [4, 25, 45, 47, 50, 54]. Thus the complex interrelationships which exist *in vivo* among the various factors determining substrate uptake by the heart may obscure the effect of any one factor examined in isolation. As an example we may compare the two situations "rest" and "rest with nicotinate". In the latter situation glucose extraction is considerably increased. The most likely explanation of this is that although arterial glucose and insulin levels are the same, FFA concentration has been lowered. Thus if the relationship between glucose extraction and insulin levels was examined alone, no simple linear correlation would be found. On the other hand, if FFA levels are also taken into consideration by using multiple regression analysis, the significant relationship between glucose uptake and *both* insulin and FFA emerges.

*Myocardial Lactate Extraction.* At rest, lactate extraction was significantly and positively related to arterial lactate concentration and significantly and negatively related to FFA extraction measured radioisotopically, on both direct correlation and multiple regression analysis.

If increased FFA extraction leads to both decreased glucose and lactate extractions, it may do so in part by inhibiting pyruvate dehydrogenase [38, 60].

*Myocardial Pyruvate Extraction.* From both direct correlation and multiple regression analyses, arterial pyruvate concentration appears to be an important factor in pyruvate extraction at rest.

Both types of analysis also suggest that increases in arterial growth hormone concentration may increase myocardial pyruvate extraction. From the known actions of growth hormone, such an effect may depend

hydrate – multiple regression analysis

| FFA Release | $C_{a-cs}$ Glucose | $C_{a-cs}$ Lactate | $C_{a-cs}$ Pyruvate | Ca Insulin | Ca Growth hormone | Ca Gluco-corticoid | Index of determination ($R^2$) | F-Ratio |
|---|---|---|---|---|---|---|---|---|
| $-0.83^{ns}$ | — | $-0.41^{ns}$ | $3.19^{ns}$ | $33.41^{a}$ | $-8.11^{ns}$ | $-24.24^{b}$ | 0.869 | $5.90^{a}$ |
| $-0.40^{ns}$ | $0.07^{ns}$ | — | $0.33^{ns}$ | $27.01^{a}$ | $-5.02^{ns}$ | $-9.67^{ns}$ | 0.918 | $10.01^{b}$ |
| — | — | — | $-0.17^{ns}$ | — | — | — | 0.818 | $21.01^{c}$ |
| $0.09^{ns}$ | $0.05^{a}$ | $0.01^{ns}$ | — | $-3.64^{b}$ | $1.33^{b}$ | $1.39^{a}$ | 0.982 | $47.22^{c}$ |
| $1.85^{ns}$ | — | $0.08^{ns}$ | $-1.01^{ns}$ | $-3.02^{ns}$ | $-2.82^{ns}$ | $8.70^{ns}$ | 0.660 | $1.72^{ns}$ |
| $1.92^{ns}$ | — | $0.09^{ns}$ | $-0.94^{ns}$ | — | $-2.56^{ns}$ | $8.25^{ns}$ | 0.656 | $2.15^{ns}$ |
| $1.06^{ns}$ | $0.03^{ns}$ | — | $1.18^{ns}$ | $5.81^{ns}$ | $3.73^{ns}$ | $-0.08^{ns}$ | 0.956 | $19.46^{c}$ |
| — | — | — | — | — | $5.19^{c}$ | — | 0.915 | $81.10^{c}$ |
| $0.04^{ns}$ | $-0.02^{ns}$ | $0.00^{ns}$ | — | $-0.66^{ns}$ | $0.23^{ns}$ | 0.00 | 0.543 | $1.06^{ns}$ |
| — | — | — | — | — | — | — | 0.397 | $10.54^{b}$ |

(5) "–" means the variable was not considered in the analysis.
(6) R is the multiple correlation coefficient.
(7) Significance is indicated by ns ($P>0.05$),[a] ($P<0.05$),[b] ($P<0{,}01$) or [c] ($P<0.001$).
(8) The best equation was chosen by, on successive elimination of independent variables, examining first the F-ratio and then the t-statistics of the partial regression coefficients.

on inhibitions of hexokinase [54] and phosphofructokinase [50, 54] with slowing down of glycolysis and less pyruvate formation.

In the multiple regression equation, insulin would decrease and glucocorticoid increase pyruvate extraction. These effects are opposite to those that these hormones have on glucose extraction. This suggests that the effects on pyruvate extraction depend on, for insulin, increased and, for glucocorticoid, decreased glycolysis and that pyruvate dehydrogenase may be rate-limiting. It seems unlikely, therefore, that activation of pyruvate dehydrogenase [17, 62] is an important effect of insulin in the human myocardium.

The regression coefficient for glucose extraction in the multiple regression equation was significant and positive. A possible explanation for this is that the pyruvate dehydrogenase complex is protected by pyruvate [53], one source of which would be the glycolytic breakdown of glucose. However, it must also be remembered that in a multiple regression analysis of the present kind, with many regression coefficients, 1 in 20 of them could be significant by chance.

### *Prolonged Exercise*

*Myocardial Glucose Extraction.* Although glucose extraction during prolonged exercise was negatively correlated with FFA extraction, the multiple regression analysis revealed only triglyceride extraction to be of possible predictive value. Fatty acid derived from plasma triglyceride could have an effect like that of FFA on myocardial glucose extraction.

There is evidence that in man exercise may be associated with augmented myocardial glucose uptake [32, 37]. Since arterial insulin concentrations fell during exercise and were unrelated to glucose extraction, even in the multiple regression equation, this would suggest that increased glucose uptake was occuring in the face of elevated growth hormone concentrations which would also tend to decrease glucose uptake [45, 47, 50, 54].

The best multiple regression equation had low predictive value. Thus factors other than the ones considered are apparently important in myocardial glucose extraction during prolonged exercise. Increased cardiac work itself could lead to the stimulation of glycolysis and glucose extraction by, for example, decreasing myocardial ATP and thereby decreasing the inhibition of phosphofructokinase [44].

*Myocardial Lactate Extraction.* The best multiple regression equation for lactate extraction during prolonged exercise was of high predictive value. Its two significant regression coefficients, those for arterial lactate and growth hormone concentrations, indicated that these factors could increase myocardial lactate extraction as direct correlation analysis also indicated. Growth hormone presumably does this by inhibiting hexokinase [54] and phosphofructokinase [50, 54] and thereby decreasing myocardial lactate formation. In this context, Regen *et al.* [54] observed a decreased lactate production from the perfused heart treated with growth hormone and cortisol.

*Myocardial Pyruvate Extraction.* Pyruvate extraction was not as well predicted by multiple regression analysis in the case of the exercise observations as it was in the case of the resting observations. Also, FFA extraction measured radioisotopically was the only factor found to be of predictive value and at rest this factor did not emerge with predictive value. The way in which FFA extraction operates is probably by inhibition of pyruvate dehydrogenase [38, 60].

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Prof. L. A. Carlson
Department of Geriatrics
University of Uppsala
Uppsala, Sweden

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