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Research Article

The free fatty acid (FFA) uptake and oxidation and the carbohydrate substrate exchange of leg muscles were studied during exercise in 14 patients with occlusive disease of the iliac or femoral arteries before and 3-6 months after reconstructive vascular surgery and in 5 healthy subjects. ^{14}C-labeled oleic acid was infused continuously at rest and during exercise at work loads of 150-400 kg-m/min. The arterial concentration of FFA was similar both at rest and during exercise in patients and controls. The patients showed a smaller increase in the fractional turnover of FFA during exercise. Leg uptake and release of FFA in terms of micromoles per liter plasma did not differ significantly either at rest or during exercise between patients and controls. FFA oxidation could not be measured at rest but exercise data showed a lower fractional oxidation of FFA ($P < 0.001$) in the patient group (53±6%) compared with the controls (84±2%). For the entire material, fractional oxidation of FFA showed a significant negative regression on the lactate/pyruvate ratio in femoral venous blood. The ventilatory respiratory quotient (RQ) and the leg muscle exchange of glucose and lactate in the patients exceeded that of the controls. When six patients were studied after reconstructive surgery, fractional oxidation of FFA had risen from a preoperative value of 47±8 to 90±10%, other data for leg […]

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ABSTRACT The free fatty acid (FFA) uptake and oxidation and the carbohydrate substrate exchange of leg muscles were studied during exercise in 14 patients with occlusive disease of the iliac or femoral arteries before and 3–6 months after reconstructive vascular surgery and in 5 healthy subjects. ^{14}C-labeled oleic acid was infused continuously at rest and during exercise at work loads of 150–400 kg-m/min. The arterial concentration of FFA was similar both at rest and during exercise in patients and controls. The patients showed a smaller increase in the fractional turnover of FFA during exercise. Leg uptake and release of FFA in terms of micromoles per liter plasma did not differ significantly either at rest or during exercise between patients and controls. FFA oxidation could not be measured at rest but exercise data showed a lower fractional oxidation of FFA ($P < 0.001$) in the patient group (53±6%) compared with the controls (84±2%). For the entire material, fractional oxidation of FFA showed a significant negative regression on the lactate/pyruvate ratio in femoral venous blood. The ventilatory respiratory quotient (RQ) and the leg muscle exchange of glucose and lactate in the patients exceeded that of the controls. When six patients were studied after reconstructive surgery, fractional oxidation of FFA had risen from a preoperative value of 47±8 to 90±10%, other data for leg muscle FFA metabolism being unchanged.

It is concluded: (*a*) that substrate catabolism by the leg muscles during exercise in these patients proceeds in excess of the simultaneous capacity to oxidize acetyl-CoA in the tricarboxylic acid cycle, and (*b*) oxidation of FFA by contracting muscle is related to the muscle cell redox state.

A preliminary report on some of the results was presented at the Karolinska Institute Symposium on "Muscle Metabolism during Exercise" held in Stockholm, Sweden, 6 September 1970 (1).

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INTRODUCTION

The free fatty acids (FFA)[1] of plasma provide a major part of the fuel used for energy production in muscle in the postabsorptive state both at rest and during mild to moderately heavy exercise. Using isotopic FFA tracers it has been possible to determine the fraction of the FFA entering muscle that is immediately oxidized by exercising muscle (fractional oxidation). During light bicycle exercise this fraction is reported to be 75–100% in healthy individuals (2, 3). During rhythmic exercise with the forearm flexor muscles the fraction was found to be inversely related to the work intensity; moderately heavy exercise yielded values in the range 40–100%. The fractional oxidation correlated negatively to the lactate/pyruvate ratio and positively to the rate of oxygen consumption (4, 5). The radioactivity not recovered as $^{14}CO_2$ during FFA-^{14}C oxidation is not retained in the muscle tissue but leaves the muscle as water-soluble metabolites, at least partly in the form of acetate and 3-hydroxybutyrate (5). These findings suggest that FFA oxidation depends not only on the FFA inflow to muscle but also on the oxygen supply and the intramuscular redox state during exercise. Both the latter variables are profoundly altered during exercise in patients with obstructive arterial disease of the lower

[1] *Abbreviations used in this paper:* A-FV, arterial and femoral venous; FFA, free fatty acids; PCA, perchloric acid; RQ, respiratory quotient.

TABLE I

Clinical Data on the Patients

Patient	Age	Height	Weight	Duration of symptoms	Claudication distance	Angiography*				Postop. study
						Com. iliac art.	Com. femoral art.	Sup. femoral art.	Pop. liteal art.	
	yr	*cm*	*kg*	*yr*	*m*					
F. N.	57	172	78	1½	90	+++	+++			
E. B.	61	184	70	1½	230	+++		+++		yes
R. J.	64	178	74	1½	90	+++	+	+	+	yes
M. E.	60	172	72	1	120	+++				
H. E.	45	178	77	1	300	++				
S. L.	51	184	70	4	50	+	+	+++	+	
B. M.	44	180	69	1	200	+++				
G. N.	61	182	86	6	300	+	++	+++	+	yes
W. B.	63	170	59	10	180	+	+	+++	++	
B. A.	48	169	77	2	115			+++	++	yes
T. C.	43	177	96	2	130	+		+++	+	
D. J.	52	176	79	14	180	+	+	+++	+	
C. K.	50	174	74	5	300	+	+	+++		yes
N. L.	50	169	75	1	230			+++		yes

* + denotes less than 75% occlusion of the vessel, ++ more than 75% occlusion, and +++ total occlusion.

extremities (6, 7). Since there is no available data on FFA metabolism during physical exertion in this condition, the present study was undertaken to further characterize the relationship between oxygen availability and FFA oxidation by exercising muscle. For this purpose a group of patients with severely impaired leg blood flow capacity due to obstructive arterial disease was studied before and after reconstructive surgery. The patients and a group of healthy volunteers were studied at rest and during a period of bicycle exercise during infusion of labeled oleic acid. The concentrations of individual FFA in plasma were measured by a gas chromatographic method and the FFA oxidation was evaluated from the production of $^{14}CO_2$ from the leg muscles.

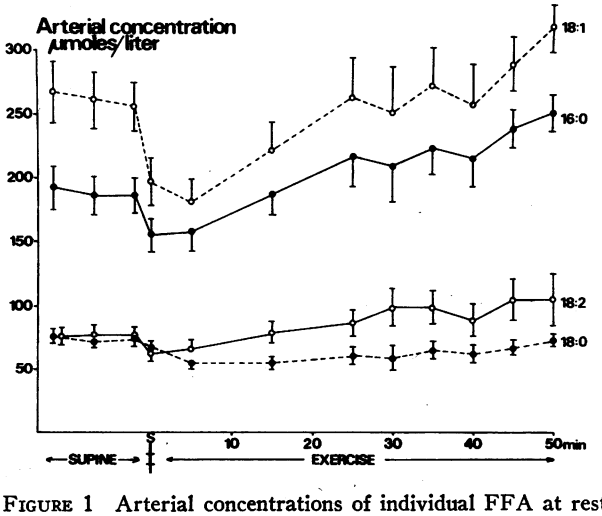

FIGURE 1 Arterial concentrations of individual FFA at rest in the supine and sitting positions and during exercise in the patient group. Data are given as mean ±SE for palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic acid (18:2).

METHODS

Subjects and procedures. 14 male patients with angiographically verified obstructions of the arteries of the lower extremities were studied. All patients developed typical symptoms of intermittent claudication during walking. Clinical data for the patients are given in Table I. Six patients were restudied 3–6 months after reconstructive surgery. No patient required insulin or oral antidiabetic agents and none was obese. A group of control subjects was also studied, consisting of five healthy male volunteers (age 40–52 yr), employed by the Stockholm fire department and participating in regular health controls. The nature, purpose, and possible risks involved in the study procedure were carefully explained to the patients and controls before obtaining their voluntary consent to participate.

The studies were performed in the morning with the subjects in the postabsorptive state after 12–14 hr of fasting. Teflon catheters were inserted percutaneously into a brachial artery, an antecubital vein, and the femoral vein of the leg to be studied. The tip of the latter catheter was placed 10–15 cm on the distal side of the inguinal ligament.

Albumin-bound oleic acid-1-^{14}C was infused intravenously at a constant rate (0.6–1.0 μCi/min) for 20 min with the subject resting in the supine position and also during the subsequent exercise period of 35–55 min. The work was performed in the upright position on a bicycle ergometer. The work load selected for the patients was the highest predetermined individual level which the patient was able to tolerate for at least 30 min. The work loads were in the range 150–400 kg-m/min (mean ±SE: 280±20 kg-m/min)

for patients, while all controls exercised at 400 kg-m/min. Blood samples for the measurement of individual FFA and FFA radioactivity, $^{14}CO_2$, oxygen content, glucose, lactate, and pyruvate concentration were obtained simultaneously from the artery and the femoral vein at timed intervals at rest in both supine and sitting position and during exercise. Expired air was collected in Douglas bags at rest and after 10 and 40 min of exercise for the determination of pulmonary oxygen uptake.

Oleic acid-1-^{14}C (SA 53.7 mCi/mmole) was obtained from the New England Nuclear Corp. (Dreieichenhain, Germany) and bound to human serum albumin as described elsewhere (4).

Analytical methods. Individual plasma FFA were measured by gas chromatography using heptadecanoic acid as an internal standard (8). In this procedure, the FFA are extracted according to Dole and Meinertz (9) and the FFA radioactivity was determined on the first heptane extract. This figure was corrected for the radioactivity of esterified fatty acids remaining in the heptane extract after alkaline extraction. The $^{14}CO_2$ content of blood was determined as described elsewhere (10). Glucose (11), lactate (12), and pyruvate (13) were measured in whole blood using enzymatic techniques. Oxygen saturation was determined spectrophotometrically (14) and hemoglobin concentration by the cyanmethemoglobin technique (15). Hematocrit was measured using a microcapillary hematocrit centrifuge and corrected for trapped plasma (16). Expired air was analyzed with the Scholander microtechnique. Labeled acetate in blood was identified by recrystallization as the *S*-benzylthiouronium salt (5).

For the various chemical analyses, the error of the method expressed as the coefficient of variation was as follows: total FFA 2.3%, oleic acid FFA-^{14}C 1.7%, glucose 1.3%, lactate 6.8% (0–1 mmoles/liter) and 3.2% (1–10 mmoles/liter), pyruvate 8%, oxygen saturation 0.9% (10–50%) and 0.4% (90–100%), hemoglobin concentration 1.2%. For glucose, lactate, pyruvate, oxygen saturation, and hemoglobin concentration duplicate analyses were made. The recovery of $^{14}CO_2$ from blood was 95±3%.

Calculations. The fractional uptake (f) of oleic acid was calculated on the basis of its arterial (A) and femoral venous (FV) radioactivity: $f = {}^{14}C-18:1_{A-FV}/{}^{14}C-18:1_A$. The uptake of oleic acid (U, micromoles/liter plasma) to the leg muscles was calculated as the product of f and the arterial plasma concentration of free oleic acid. Release of oleic acid (R, micromoles/liter) was estimated as the difference between U and the net arteriovenous difference for unlabeled oleic acid across the leg $(18:1_{A-FV}): R = U - 18:1_{A-FV}$. The fractional oxidation of the oleic acid (f_{ox}) was determined as: $f_{ox} = 100 \times ({}^{14}CO_{2\,FV-A})/({}^{14}C-18:1_{A-FV})$ per cent after hematocrit correction. The turnover rate of oleic acid was calculated as the amount of radioactivity infused per unit time divided by the oleic acid specific activity. The fractional turnover of oleic acid was calculated as its turnover divided by its arterial concentration. The plasma volume was not determined and the fractional turnover is therefore expressed in terms of liters per minute.

Data in the text, tables, and figures are given as mean ±SE. Standard statistical methods have been employed in analyzing the data, using the paired *t* test when applicable. Comparisons of exercise data between patients and controls were made in part by analysis of variance using linear combinations of means as described by Snedecor and Cochran (17).

RESULTS

Preoperative results

Data on heart rate and pulmonary oxygen uptake at rest and during exercise in the patient and control groups are given in Table II. All patients developed mild to moderate symptoms of fatigue and/or pain from the afflicted leg during exercise. Heart rate at rest was slightly higher in the patient group ($P < 0.05$) and rose more in response to exercise in the patients ($P < 0.001$), than in the controls despite the lower average work intensity in the former group. Basal oxygen uptake did not differ between the groups but the controls showed a 14–18% higher pulmonary oxygen uptake during exercise ($P < 0.01$). The ventilatory RQ did not differ at rest but was significantly higher in the patient group during exercise ($P < 0.01$).

Arterial concentration and turnover of FFA. Only the four main FFA (palmitic, stearic, oleic, and linoleic acid) were determined and the total FFA concentration represents the sum of these four acids. The arterial FFA

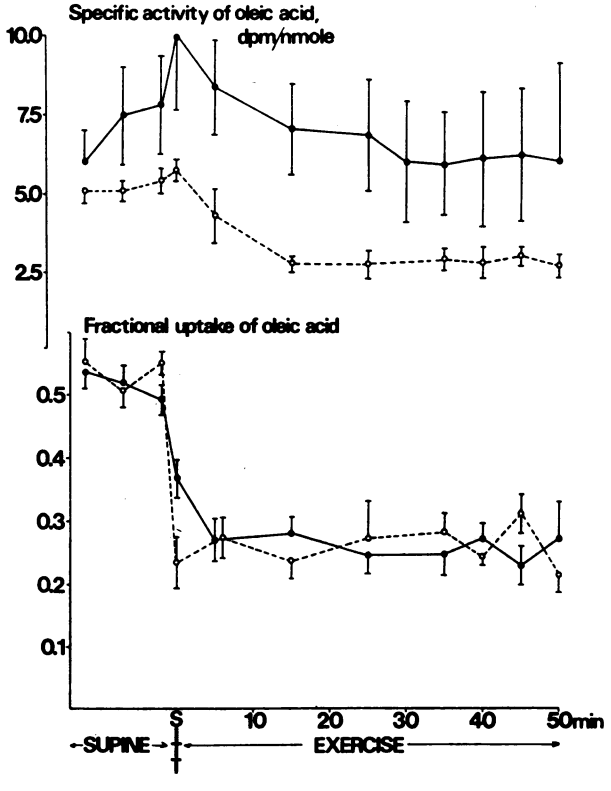

FIGURE 2 Specific activity of oleic acid in arterial plasma and leg fractional uptake of oleic acid at rest in the supine and sitting positions and during exercise for patients (●——●) and controls (○- - -○). Data are given as mean ±SE. The difference in SE of oleic acid specific activity between the groups is due to the fact that the rate of infusion of radioactivity differed between the patients but was the same in all control subjects.

TABLE II

Data on Circulatory and Metabolic Adaptation at Rest and during Exercise in Patients (P) and Control Subjects (C)

		Rest		Exercise					
		Supine	Sitting	5 min	10 min	15 min	25 min	35 min	45 min
Heart rate, *beats/min*	P	72±3	—	98±5	105±5	110±5	116±5	120±6	135±6
	C	55±5	—	84±8	88±7	90±10	92±9	91±9	98±9
Pulmonary oxygen uptake, *ml/min*	P	256±13	—	—	790±49	—	—	869±54	—
	C	267±18	—	—	935±71	—	—	995±76	—
Respiratory quotient	P	0.77±0.02	—	—	0.82±0.01	—	—	0.81±0.02	—
	C	0.75±0.02	—	—	0.77±0.01	—	—	0.76±0.01	—
A-FV oxygen difference, *ml/liter*	P	70.4±6.4	136.6±8.3	166.6±7.1	—	161.5±5.5	161.8±6.3	164.8±5.9	167.0±12.3
	C	81.4±12.5	110.5±13.1	157.3±4.9	—	159.7±6.8	156.6±4.4	153.5±3.7	153.5±4.3
FV oxygen saturation, %	P	59.2±3.3	26.9±2.8	15.3±2.0	—	15.9±2.0	15.8±2.6	14.6±1.9	11.4±3.5
	C	53.2±6.5	38.8±7.5	18.7±1.2	—	19.1±2.1	18.4±1.9	20.0±1.3	18.1±1.4
Arterial FFA, *μmoles/liter*	P	598±43	481±39	456±42	—	544±46	626±69	660±66	701±52
	C	582±40	500±24	475±22	—	635±26	748±72	731±79	726±86
Arterial glucose, *mmoles/liter*	P	3.96±0.13	3.89±0.13	3.96±0.13	3.93±0.10	3.95±0.11	4.02±0.11	4.04±0.12	4.12±0.20
	C	4.20±0.08	4.06±0.16	4.24±0.09	4.21±0.11	4.42±0.11	4.34±0.09	4.21±0.08	4.28±0.10
A-FV glucose, *mmoles/liter*	P	0.12±0.05	0.16±0.05	0.27±0.04	0.26±0.06	0.31±0.05	0.31±0.04	0.43±0.08	0.38±0.04
	C	0.17±0.03	0.09±0.13	0.13±0.04	0.17±0.08	0.33±0.04	0.27±0.09	0.29±0.07	0.32±0.06
Arterial lactate, *mmoles/liter*	P	0.66±0.05	0.78±0.06	1.90±0.19	1.88±0.18	1.81±0.21	1.83±0.17	1.92±0.21	1.72±0.22
	C	0.57±0.06	0.65±0.07	0.93±0.21	0.67±0.10	0.80±0.10	0.78±0.13	0.77±0.10	0.77±0.09
A-FV lactate, *mmoles/liter*	P	−0.24±0.04	−0.13±0.02	−0.51±0.13	−0.45±0.08	−0.33±0.09	−0.49±0.11	−0.52±0.17	−0.56±0.22
	C	−0.18±0.03	−0.13±0.05	−0.09±0.08	−0.13±0.08	−0.02±0.09	−0.12±0.13	−0.06±0.05	−0.04±0.03
Arterial pyruvate, *μmoles/liter*	P	73±4	78±5	109±8	115±12	107±11	110±12	117±13	108±5
	C	66±10	65±4	60±7	66±8	73±3	77±8	80±7	68±7
A-FV pyruvate, *μmoles/liter*	P	−6±3	−10±8	1±8	11±11	7±7	7±8	1±7	−2±7
	C	6±5	−5±6	9±8	22±7	15±12	1±5	5±3	11±10
Femoral venous L/P ratio	P	14.5±1.5	14.4±1.2	21.5±2.1	21.6±2.2	18.9±1.6	18.1±1.2	19.5±2.2	20.6±2.1
	C	11.2±2.0	13.5±1.5	17.5±7.4	11.1±0.8	8.6±0.6	9.5±1.0	9.4±1.0	10.1±0.7

concentration at rest was the same in the patients and the controls and the changes during exercise were similar in both groups (Table II). In the patient group the concentrations of all four individual FFA fell significantly at the transition from the supine to the sitting position ($P < 0.01$, Fig. 1). The concentrations of palmitic, oleic, and linoleic acid then rose during exercise ($P < 0.01$) and reached values 20–30% above the resting value measured in the supine position. The concentration of stearic acid, on the other hand, decreased during the first part of the exercise period, being significantly lower than in the sitting position after 5 and 15 min of exercise ($P < 0.001$). After 45 min of exercise, the stearic acid level was still below that observed during the supine rest period ($P < 0.01$). Results for the control subjects with regard to individual FFA did not differ significantly from the findings in the patient group.

The specific activity of oleic acid had not reached a constant level at the first observation after the start of the infusion in the resting state in the patients (Fig. 2). However, the following two values for oleic acid specific activity did not differ significantly and only these were used for calculating the oleic acid turnover at rest. The fall in oleic acid concentration on changing from the supine to the sitting position was associated with a corresponding increase in its specific activity ($P < 0.05$) and there was no change in the circulating level of radioactivity and, hence, neither in the fractional turnover of oleic acid. During exercise the oleic acid specific activity decreased from the peak observed in the sitting position to a constant level that was reached after about 30 min in the patients and after approximately 15 min in the control subjects. The plateau value of specific activity used for calculating oleic acid turnover during exercise was the mean of at least three measurements in each subject.

The turnover of oleic acid at rest was the same in patients and controls and correlated to the arterial oleic acid concentration (Table III, Fig. 3). The difference between patients and controls regarding fractional turnover of oleic acid at rest (0.97 compared with 1.07, Table III) was statistically significant ($P < 0.05$). However, variations in plasma volume (not measured) may have contributed to this difference. The turnover of oleic acid increased more during exercise than did the arterial concentration, so that the fractional turnover was higher than at rest ($P < 0.01$). The increase in fractional turnover of oleic acid induced by exercise was greater in the control subjects than in the patients ($P < 0.005$). The regression of turnover of oleic acid on its arterial concentration persisted during exercise in both groups ($Y = 0.91X + 76$, $P < 0.01$ for the patients; $Y = 1.51X + 57$, $P < 0.01$ for the controls).

Leg uptake and release of FFA. The arterial-femoral venous (A-FV) FFA difference at rest was 23±26 μmoles/liter in the patient group and 54±20 μmoles/liter in the controls. Neither of these values differs significantly from zero. During exercise a significant A-FV difference of FFA ($P < 0.001$) was observed in both groups, amounting to 89±19 μmoles/liter in the patients and 82±13 μmoles/liter in the controls.

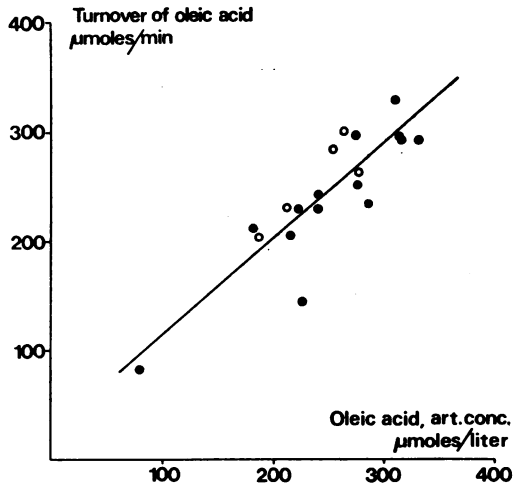

FIGURE 3 Turnover of oleic acid at rest in relation to its arterial concentration in patients (●) and controls (○). ($Y = 0.89X + 23$, $n = 19$, $r = 0.88$, $P < 0.001$).

The fractional uptake of oleic acid in the leg at rest was similar in both groups (Fig. 2). On changing to the sitting position, the fractional uptake fell in both groups ($P < 0.001$), the decrease being significantly greater in the controls ($P < 0.005$). The patient group showed a further decrease during the initial phase of exercise ($P < 0.02$), so that the fractional uptake of oleic acid during exercise became similar in the two groups and amounted to about 50% of the resting value observed in the supine position.

The uptake of oleic acid, calculated on the basis of uptake of the tracer FFA and expressed in micromoles/liter plasma, underwent changes similar to those observed for its fractional uptake (Fig. 4). A decrease was thus noted in both groups when the position was changed from

TABLE III

Turnover of Oleic Acid at Rest and during Exercise in Patients and Controls

	Turnover		Fractional turnover	
	Rest	Exercise	Rest	Exercise
	μmoles/min		*liter/min*	
Patients, preop.	239±18	317±31*‡	0.97±0.03‡	1.27±0.08*‡
Patients, postop.	232±16	371±50*	0.91±0.05	1.21±0.10*
Control subjects	256±18	499±44*	1.07±0.03	1.72±0.03*

* Differs significantly from the corresponding value at rest ($P < 0.01$–0.001).

‡ Differs significantly from the corresponding value for the control subjects ($P < 0.05$–0.001).

TABLE IV

Recovery of Radioactivity from the Oxidation of Oleic Acid-1-^{14}C

Exp.	A-FV oleic acid-^{14}C (1)	FV-A $^{14}CO_2$ (2)	FV-A PCA-^{14}C (3)	Recovery $\frac{(2)^* + (3)^*}{(1)^*} \times 100$	Acetate-^{14}C‡
	dpm/ml blood	*dpm/ml blood*	*dpm/ml blood*	%	per cent of FV PCA-^{14}C
1	101	38	48	85	—
2	277	232	45	100	—
3	244	164	36	82	66, 69§
4	212	153	77	109	39

* Refer to column numbering.

‡ These analyses were made on PCA extract from 5 to 10 ml femoral venous blood. Acetate was recrystallized twice as the *S*-benzylthiouronium salt after addition of 3 mmoles of carrier acetate (5). The final specific activity of the salt was 0.5–1.5 dpm/mg.

§ Duplicate analyses.

supine to sitting ($P < 0.01$). It should be noted, however, that the values calculated for the sitting position are based on a rapidly changing arterial FFA concentration and should thus be interpreted with caution. The mean uptake during exercise was 50–60% of that observed at rest in the supine position.

Similar changes were also found in the release of oleic acid from the leg (Fig. 4). A fall was observed on changing the resting position ($P < 0.01$). During exercise the release of oleic acid amounted to about 50% of the simultaneous uptake, as compared with 75–80% at rest.

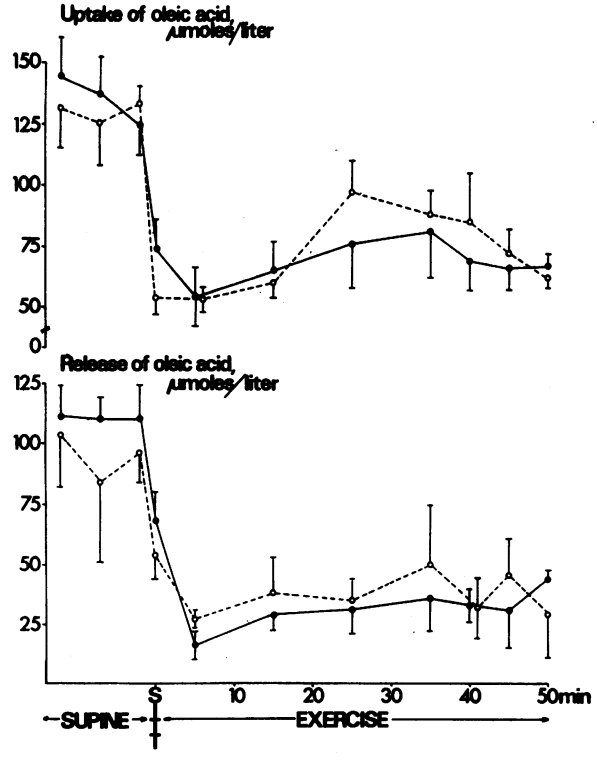

FIGURE 4 Leg uptake and release of oleic acid at rest in the supine and sitting positions and during exercise for patients (●——●) and controls (○- - - -○). Data are given as mean ±SE.

Oxidation of FFA. It was not possible to measure the oxidation of the labeled oleic acid at rest due to the slow turnover of the bicarbonate pool of the leg tissues. During the initial phase of exercise, the production of $^{14}CO_2$ in the leg often exceeded the simultaneous uptake of labeled oleate, indicating a washout of these sluggish pools, in which label had accumulated during infusion in the resting state. The production of $^{14}CO_2$ reached a constant level after about 25–30 min of exercise and the fractional oxidation of oleic acid was estimated from the data obtained during the last 20 min of exercise. It amounted to 53±6% in the patient group and 84±2% in the controls ($P < 0.001$) and showed a significant negative regression on the lactate/pyruvate ratio measured in femoral venous blood ($r = -0.83$, $P < 0.001$, Fig. 5).

In four of the experiments in the patient group the release of radioactive water-soluble metabolites was determined by measuring the FV-A difference of radioactivity in a neutralized perchloric acid (PCA) extract of the blood. The sum of $^{14}CO_2$ production and PCA radioactivity in these experiments amounted to 94±6% of the FFA-^{14}C uptake (Table IV). Radioactive acetate was identified in the femoral-venous PCA extract in two of the experiments, making up 39 and 68% of femoral-venous PCA extractable radioactivity.

Oxygen uptake and carbohydrate metabolism (Table II). The A-FV oxygen difference did not differ between the two groups at rest but rose more in the patients during exercise ($P < 0.05$). The arterial level of glucose did not differ at rest and rose slightly and approximately equally in both groups during exercise. The A-FV difference for glucose was similar in the two groups at rest and grew gradually during the exercise period in both

groups, though for the entire period of exercise the difference was greater in the patient group ($P < 0.02$). The arterial concentrations and the A-FV differences for lactate and pyruvate at rest were similar for the two groups. Arterial levels and A-FV differences both rose more markedly in the patient group in response to exercise ($P < 0.001$). The femoral venous lactate/pyruvate ratio did not differ between the two groups at rest but rose to higher values during exercise in the patient group ($P < 0.001$).

Postoperative results

Six of the patients were restudied 3–6 months after vascular surgery, when the symptoms of fatigue and claudication on walking had disappeared. The postoperative study was performed at the same work load as in the preceeding study and the infusion of labeled FFA and the blood sampling were conducted in the same way. No symptoms of fatigue or pain occurred during the exercise period. The results are summarized in Table V. Heart rate during exercise was lower postoperatively ($P < 0.001$), while the pulmonary oxygen uptake and the RQ were unchanged. The arterial FFA level as well as the uptake and release of FFA by the leg had not changed significantly after reconstructive surgery. However, the fractional oxidation of oleic acid in the leg during exercise increased from a preoperative value of 47±8 to 90±10% in the postoperative study ($P < 0.001$). A simultaneous decrease in the femoral venous lactate/pyruvate ratio was observed in five of the six patients. In the regression between fractional oxidation and the lactate/pyruvate ratio, the postoperative measurements therefore tended to move upwards to the left along the previously observed regression line (Fig. 6).

The A-FV oxygen difference was unchanged at rest but rose less during the postoperative exercise period ($P < 0.01$). The arterial level of glucose was slightly higher both at rest and during exercise ($P < 0.01$) in the postoperative study, but the A-FV glucose difference was not significantly changed. The arterial levels of lactate and pyruvate during exercise were mainly unchanged during exercise postoperatively but the FV-A lactate difference was significantly lower ($P < 0.001$). The FV-A difference for pyruvate was not significantly altered postoperatively. The average lactate/pyruvate ratio was slightly lower after the operation both at rest ($P < 0.05$) and during exercise ($P < 0.01$).

DISCUSSION

The work intensity at which the patients exercised was chosen as the highest they could sustain for 30–40 min, The control subjects, on the other hand, all exercised at 400 kg-m/min, which was the highest work intensity employed in the patient group. This difference in work

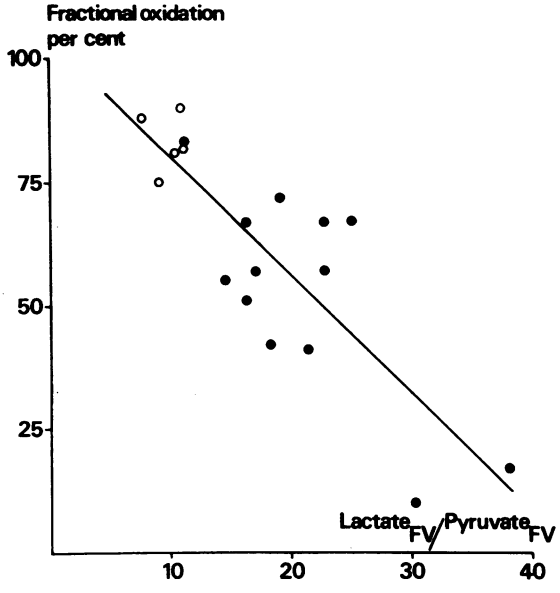

FIGURE 5 Fractional oxidation of oleic acid during exercise in relation to the femoral venous lactate/pyruvate ratio in patients (●) and controls (○) ($Y = -2.38X + 103$, $r = 0.83$, $P < 0.001$).

load is reflected in the slightly higher pulmonary oxygen uptake in the control group. The differences between the two groups for all other metabolic variables measured were in the opposite direction to what would be expected on the basis of the difference in work inten-

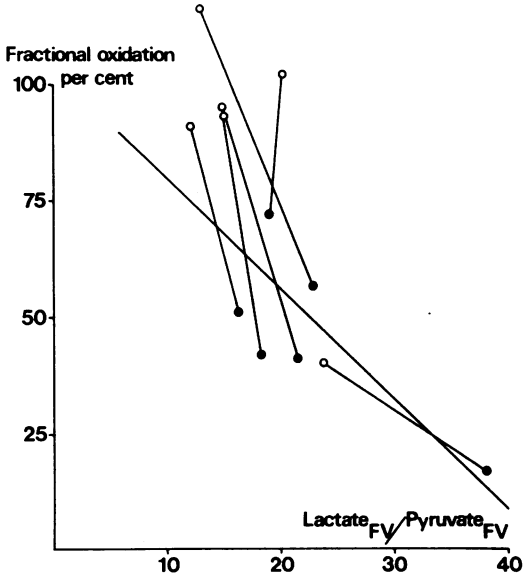

FIGURE 6 Fractional oxidation pre- (●) and postoperatively (○) in relation to the femoral venous lactate/pyruvate ratio. The solid line represents the regression of fractional oxidation on femoral venous lactate/pyruvate ratio seen in preoperative patients and control subjects (Fig. 5).

TABLE V

Pre- and Postoperative Data Regarding Circulatory and Metabolic Adaptation at Rest and during Exercise

		Rest		Exercise					
		Supine	Sitting	5 min	10 min	15 min	25 min	35 min	45 min
Heart rate, *beats/min*	Pre	69±5	—	102±9	109±10	116±10	124±9	131±8	149±4
	Post	71±5	—	100±5	106±6	112±7	116±8	122±8	118±8
Pulmonary oxygen uptake, *ml/min*	Pre	245±15	—	—	802±73	—	—	1046±65	—
	Post	221±9	—	—	796±106	—	—	902±96	—
Respiratory quotient	Pre	0.75±0.03	—	—	0.81±0.02	—	—	0.82±0.03	—
	Post	0.73±0.02	—	—	0.81±0.02	—	—	0.81±0.01	—
A-FV oxygen difference, *ml/liter*	Pre	59.6±8.0	139.2±12.6	154.2±9.4	—	161.7±9.1	160.5±10.9	165.5±9.6	174.1±14.3
	Post	62.2±11.4	131.5±10.3	151.8±8.1	—	156.9±9.3	160.0±8.0	157.6±4.2	161.7±9.1
FV oxygen saturation, %	Pre	64.8±4.3	24.8±3.8	16.0±3.3	—	14.4±2.9	12.7±4.0	11.3±2.5	9.7±4.2
	Post	62.5±5.0	27.1±3.3	19.7±2.0	—	16.4±0.9	15.2±1.2	17.4±1.6	12.7±0.7
Arterial FFA, *μmoles/liter*	Pre	613±46	480±50	489±57	—	588±52	694±73	728±61	712±84
	Post	598±26	519±25	480±24	—	589±40	775±60	724±92	734±65
Fractional uptake of oleic acid	Pre	0.48±0.02	0.34±0.01	0.26±0.07	—	0.25±0.03	0.26±0.03	0.24±0.05	0.22±0.02
	Post	0.47±0.06	0.36±0.03	0.23±0.02	—	0.22±0.02	0.22±0.02	0.23±0.02	0.25±0.03
Arterial glucose, *mmoles/liter*	Pre	4.04±0.18	3.85±0.12	3.92±0.10	4.03±0.11	3.92±0.15	3.90±0.09	4.00±0.09	4.15±0.16
	Post	4.22±0.09	4.24±0.12	4.33±0.09	4.34±0.09	4.41±0.09	4.50±0.09	4.26±0.08	4.37±0.09
A-FV glucose, *mmoles/liter*	Pre	0.27±0.06	0.12±0.12	0.28±0.05	0.29±0.11	0.36±0.11	0.29±0.08	0.46±0.11	0.44±0.06
	Post	0.16±0.04	0.31±0.10	0.32±0.07	0.33±0.03	0.27±0.04	0.38±0.05	0.25±0.04	0.42±0.06
Arterial lactate, *mmoles/liter*	Pre	0.63±0.07	0.80±0.11	1.74±0.21	1.78±0.17	1.64±0.19	1.75±0.12	2.09±0.28	1.94±0.30
	Post	0.64±0.08	0.83±0.07	1.56±0.26	1.66±0.33	1.71±0.29	1.63±0.31	1.84±0.34	1.53±0.30
A-FV lactate, *mmoles/liter*	Pre	−0.23±0.06	−0.09±0 03	−0.57±0.14	−0.46±0.10	−0.39±0.12	−0.62±0.16	−0.47±0.30	−0.64±0.40
	Post	−0.24±0.07	−0.14±0.04	−0.23±0.08	−0.30±0.14	−0.14±0.18	−0.14±0.09	−0.26±0.16	−0.12±0.20
Arterial pyruvate, *μmoles/liter*	Pre	65±7	74±2	105±10	117±10	89±13	110±15	109±8	115±5
	Post	71±7	77±9	97±5	106±7	104±15	104±7	117±16	96±8
A-FV pyruvate, *μmoles/liter*	Pre	1±2	1±7	−5±10	−1±10	−18±13	−9±12	−6±6	6±10
	Post	−5±9	−1±6	1±10	−6±4	2±13	−3±3	−7±12	−12±7
Femoral-venous L/P ratio	Pre	13.9±1.7	14.3±2.4	20.8±1.7	21.7±2.2	19.5±1.7	20.2±1.0	20.0±3.7	22.7±3.3
	Post	11.4±0.7	13.7±1.3	19.8±2.8	16.8±3.3	18.6±4.8	16.0±2.6	20.5±2.6	16.1±1.5

sity. It is thus apparent that the somewhat lower work load in the patient group does not affect the validity of comparisons between the two groups.

Despite the somewhat lower work intensity for the patients, their increase in heart rate during exercise was higher. This may be due to a difference in physical fitness between the two groups but, to some extent it may also be connected with the fact that all the patients complained of fatigue and pain in the leg muscles during the latter part of the exercise period. None of these symptoms occurred in the control groups and muscle pain during submaximal exercise has been shown to induce an increase in heart rate (18). This explanation for the greater increase in heart rate among the patients during exercise is further supported by the observation that the rate was lower at the same work intensity in the six who were restudied after reconstructive surgery, when these patients experienced no pain or symptoms of fatigue.

The limitation in blood flow through the diseased legs of the patients was compensated for by an increased extraction of oxygen from the blood during exercise, resulting in a lower femoral venous oxygen saturation compared with the controls. Since the leg blood flow was not measured it is not possible to determine if this rise in oxygen extraction was large enough to fully compensate for the reduction in blood flow. It seems reasonable to assume, however, that a relative lack of oxygen in fact limited the performance of at least some of the patients in whom the femoral venous oxygen saturation decreased to values below 10%.

FFA metabolism. The increase in fractional turnover of oleic acid elicited by the exercise is most likely due to an augmented utilization of FFA in the working muscle. The uptake of FFA by exercising muscle is linearly related to the FFA inflow, i.e. plasma flow times arterial plasma concentration (19). The latter was the same in patients and controls and the lower fractional turnover of oleic acid in the patients was therefore probably a consequence of a reduced leg blood flow and, hence a decreased inflow of FFA to the exercising muscles.

The oxidation of FFA by the exercising leg muscle was severely impaired in the patients in comparison with the controls. This may, at least in some of the patients have been due to a lack of oxygen in the muscle cells as discussed above. However, the uptake of both oxygen and FFA, calculated per liter blood, was the same for the patients and the controls. Therefore the reduction of leg blood flow in the patient group decreased the supply of oxygen and FFA to the same extent. Thus the lower fractional oxidation cannot be explained on the basis of a disturbed balance between the availability of FFA and oxygen. The impaired total supply of oxygen restricts the oxidative phosphorylation so that the constant demand for adenosine triphosphate (ATP) regeneration imposed by the exercise must be covered by a larger contribution from glycolysis. This increased breakdown of carbohydrate may have been elecited by a low energy charge of the adenine nucleotides (20) as a consequence of the reduced oxidative phosphorylation, and it was reflected by a higher ventilatory RQ, a more efficient uptake of glucose, and a higher production of lactate and pyruvate in the patient group. The increased availability of pyruvate for oxidative decarboxylation will then increase the production of acetyl-CoA from carbohydrate and it seems reasonable to explain the low fractional oxidation in terms of the imbalance between an increased production of acetyl-CoA and a low oxidative phosphorylation. This hypothesis is consistent with the correlation observed between the fractional FFA oxidation and parameters of carbohydrate metabolism such as the venous-arterial lactate difference (4) and the lactate/pyruvate ratio (present study).

The fate of the ^{14}C-labeled FFA not appearing as $^{14}CO_2$ is not readily determined in a continuous infusion study of the current type due to the low levels of radioactivity obtained. However, in the four patients who received the highest rate of oleic acid-^{14}C infusion (1 μCi/min) it was possible to demonstrate that the FFA label not recovered as $^{14}CO_2$ left the leg muscles quantitatively in the form of perchloric acid extractable water-soluble metabolites. The presence of labeled acetate in femoral-venous blood was demonstrable in two subjects (Table IV). The phenomenon of partial FFA oxidation during muscular exercise has been studied more extensively in healthy individuals during different types of forearm exercise. By infusing FFA-^{14}C into the brachial artery it was possible to establish that the FFA fractional oxidation was related to the work intensity, complete oxidation being achieved at low work loads and values of 40–60% being reached during strenuous exercise (4, 5). In the latter studies acetate and 3-hydroxybutyrate were identified among the FFA oxidation products leaving the muscle. These findings support the notion that in healthy subjects performing strenuous exercise as well as in the present patients with impaired blood flow capacity of the legs exercising at a light work load, the restriction of fatty acid oxidation occurs at the level of acetyl-CoA utilization.

It should be noted that a functional impairment of the mitochondria or a decrease in their number per gram muscle would have similar metabolic effects. With regard to the fractional oxidation of FFA, it is noteworthy that the current results agree with findings for paretic muscle during electrically induced exercise (21). In the latter study the fractional oxidation of FFA was low despite an adequate supply of oxygen. The muscle tissue in both these patient groups lacks the training effect of normal physical activity and it is possible that this is associated with changes in the mitochondrial apparatus that are partly responsible for the observed im-

pairment of FFA oxidation capacity. Further support for this formulation may be obtained from comparisons with the contrasting state of physically trained muscle, which has a greater number of mitochondria per gram muscle and a higher capacity for the oxidation of fatty acids (22).

The change from supine to sitting position at rest was associated with changes in the FFA metabolism that cannot be fully understood on the basis of the present data. The blood samples obtained in the sitting position were drawn 1–2 min after the change in posture, during which time the arterial FFA concentration fell 15–20%. The arterial level of radioactivity did not change and the fractional turnover of oleic acid was thus not influenced, indicating that the fall in the arterial concentration of FFA was due to a decrease in the influx of FFA to the circulation. This is most readily explained on basis of a diminished blood flow through subcutaneous adipose tissue as a result of increased sympathetic activity elicited by the change in body posture. An augmented sympathetic tone affects the lipolytic process in two ways, triglyceride hydrolysis being stimulated but the FFA formed being trapped in the adipose tissue as a consequence of vasoconstriction and decreased blood flow (23). The simultaneous decrease in the fractional uptake of oleic acid is more difficult to interpret. One would rather have expected a change in the opposite direction since the fractional FFA uptake correlates negatively to the arterial FFA level as well as to the blood flow (19). Repeated samples were otbained in the sitting position in a few experiments and the results indicate that the fractional uptake of oleic acid fell only transiently, returning to the initial level within 5–10 min. A redistribution of the blood flow through the leg or a change in the area of the capillary bed may well serve to explain this transient change in fractional FFA uptake. An intact circulation and vasomotor regulation seems to be a prerequisite for this phenomenon since the decrease in fractional uptake of oleic acid was greater in the control subjects than in the patients.

Many authors have described an initial fall in the plasma FFA concentration during exercise (6, 24). In the present study there was no further decrease in FFA from the level reached when the subjects sat up. This may have been an effect of the relatively light work intensity but the possibility remains that the earlier conclusions were based on a decrease of FFA from levels observed during supine rest and that this decrease actually occurred before exercise started.

The increase in plasma FFA during exercise was not reflected by the concentration of stearic acid, which throughout the exercise period remained below the level observed at rest. The composition of plasma FFA thus changes considerably during exercise. This finding underscores the importance of calculating FFA specific activities on the basis of the concentration of the individual acid actually used as a tracer and not on total plasma FFA concentrations (25).

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