Oxidation of Free Fatty Acids by Skeletal Muscle during Rest and Electrical Stimulation in Control and Diabetic Dogs* (33924)

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Since the demonstration of the ability of skeletal muscle to oxidize plasma free fatty acids (FFA) *in vivo* (1–3), a number of studies dealt with the contribution of FFA to the energy metabolism of skeletal muscle (4–7) mostly during exercise. Much less information is available concerning the metabolism of the resting skeletal muscle under *in vivo* conditions. The present study represents an effort to investigate the uptake and oxidation of FFA by skeletal muscle in the same animal during rest and during stimulation and to compare the data of control animals with those obtained in alloxan-diabetic dogs. In addition, the effects of 2, 4-dinitrophenol (DNP) administration were also studied in the control animals.

Materials and Methods. The experiments were performed on 10 male, mongrel dogs weighing 19–26 kg. Food was withheld for about 18 hr prior to the experiment. In five of the animals, diabetes was produced by the intravenous injection of 80 mg/kg of alloxan 2–3 days prior to the experiment. All five animals had fasting blood sugar levels of more than 240 mg/100 ml.

The animals were anesthetized with sodium pentobarbital and the right femoral vein was cannulated distal to the profunda femoris veins. The cannula was connected to a rotameter with a saline-containing tubing as described previously (8). During the measurement of blood flow and the sampling of blood, the femoral vein was occluded proximally from the profunda femoris veins, thus diverting the blood flow from the latter veins towards the rotameter. Following the blood flow estimation and the removal of simultaneous blood samples from the profunda femoris veins and the femoral artery (through a side branch), the femoral occlusion was discontinued and venous blood was returned to the animal (8). No anticoagulant was used in the animals.

In order to calculate the oxidation of FFA from the production of $^{14}CO_2$ by an anatomical region, the tissue and plasma bicarbonate pools should be in equilibrium with regard to $^{14}CO_2$. This is difficult to achieve in the resting muscle and requires a very long infusion of labeled FFA. To facilitate equilibration, 50 μCi of $NaH^{14}CO_3$ was administered intravenously over 10 min, followed by an infusion of albumin-bound 1-^{14}C-palmitate and 9-10-^{3}H-oleate for the entire duration of the experiment. Ninety min of the labeled FFA infusion resulted in constant arterial levels of $^{14}CO_2$. Three pairs of control blood samples were then taken at 20-min intervals, followed by direct electrical stimulation of the muscles (6–8 V, 6/sec frequency, and 20 msec duration) for 20 min. Two pairs of blood samples were obtained during stimulation (in the middle and at the end of the stimulation period). One hr of recovery was allowed in the nondiabetic dogs; two pairs of blood samples were taken during this phase (at 40 and 60 min). An infusion of 2, 4-dinitrophenol (2.5 mg/min) was then administered for 50 min, and two more pairs of blood samples were taken (at midpoint and end of the infusion). The dosage of DNP was selected on the basis of previous experiments (9). In diabetic dogs, the recovery phase following electrical stimulation, lasted for 90 min. Data are expressed as means ± SEM for each phase of the study. The statistical significance between the means was calculated using the Student's *t* test.

Blood samples were anticoagulated with

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heparin *in vitro* and centrifuged at 4°. Plasma FFA was determined in duplicate by the method of Dole and Meinertz (10), blood CO_2 by using the AutoAnalyzer, blood $^{14}CO_2$ by the method of Passman *et al.* (11). Lactate, glycerol, and β-hydroxybutyrate were determined in whole blood which was precipitated immediately after removal from the animal (12). Plasma lipids were separated by thin-layer chromatography (13) with the solvent system of hexane, ethyl ether, and glacial acetic acid (156:40:4, v/v/v). The FFA spot was scraped into counting vials, 10 ml of Bray's solution was added and the radioactivity was assayed in a Packard liquid scintillation spectrometer. Radioactivity was expressed in terms of dpm, after allowing for quenching and counting efficiency.

The data presented were obtained by use of the calculations (14) in Eq. (1) through (6).

$$\text{Extraction of labeled fatty acid (percentage of arterial level)} = \frac{\text{FFA}_a\ (\text{dpm}) - \text{FFA}_v\ (\text{dpm})}{\text{FFA}_a\ (\text{dpm})} \times 100, \quad (1)$$

where the subscripts a and v indicate arterial or venous (profunda femoris) plasma.

$$\text{FFA uptake } (\mu\text{eq/min}) = \frac{[\text{FFA}_a{}^{14}\text{C (dpm)} - \text{FFA}_v{}^{14}\text{C (dpm)}] \times \text{plasma flow}}{\text{FFA}_a{}^{14}\text{C SA}}. \quad (2)$$

$$\text{FFA oxidation } (\mu\text{eq/min}) = \frac{[{}^{14}\text{CO}_{2_v}\ (\text{dpm}) - {}^{14}\text{CO}_{2_a}\ (\text{dpm})] \times \text{plasma flow}}{\text{FFA}_2{}^{14}\text{C SA} \times [1 - (\text{HCT}/100)]}. \quad (3)$$

$$\text{FFA release } (\mu\text{eq/liter}) = \text{FFA}_v\ (\mu\text{eq/liter}) - \left[\frac{\text{FFA}_v{}^{14}\text{C (dpm)}}{\text{FFA}_a{}^{14}\text{C (dpm)}} \times \text{FFA}_a\ (\mu\text{eq/liter})\right]. \quad (4)$$

$$\text{FFA flux (15) } (\mu\text{eq/min}) = \frac{F}{\overline{SA}}, \quad (5)$$

where F = continuously infused radio-FFA (dpm/min); $\overline{SA}$ = arterial FFA specific activity (dpm/μeq).

$$\text{CO}_2 \text{ production derived from FFA oxidation } (\%) = \frac{\text{FFA oxidation} \times 17}{\text{CO}_2 \text{ production}} \times 100. \quad (6)$$

Results. Control dogs. The following changes were observed during electrical stimulation of the muscles (Table I): Blood flow increased considerably, and the extraction of FFA decreased. The uptake of FFA per unit time increased in four of the five dogs studied. The arterial FFA level did not change significantly; however, oxidation of FFA greatly increased. About 36% of the removed FFA was oxidized during rest, and 60% during stimulation. Stimulation of the muscles had only local but no systemic effects, as indicated by the unchanged arterial levels of glucose and lactate and the unchanged FFA flux. Lactate output increased but the increase was not statistically significant. A significant glucose uptake was demonstrable during rest, while no significant removal was seen during stimulation.

During recovery, blood flow returned to control level, FFA extraction increased, oxidation of FFA was very low,—accounting for only 12% of the uptake, — and a significant glucose uptake was found.

Administration of DNP raised the turnover of FFA in all animals, although the change was not statistically significant due to the large individual variations. FFA uptake and oxidation were elevated and ⅔ of the removed FFA was oxidized, accounting for approximately ⅓ of the CO_2 production.

Diabetic dogs. Arterial FFA and FFA flux were greatly elevated in the diabetic dogs (Table II) as compared to the control animals, and again showed no significant changes during stimulation. FFA extraction was lower and both uptake and oxidation were higher in diabetic than in control animals. During muscle stimulation, FFA extraction further decreased and oxidation (but not up-

TABLE I. Changes in Skeletal Muscle Metabolism during Electrical Stimulation, Recovery, and 2,4-Dinitrophenol Administration in Control Dogs ($N = 5$).

	Rest	Stimulation	Recovery	DNP
Blood flow (ml/min)	21.6 ± 5.0[a]	76.5 ± 18.1[b,c]	18.9 ± 6.1	55.8 ± 15.6[f]
Art. FFA (μeq/liter)	545 ±101	597 ±130	567 ±100	599 ±107
^{14}C-palmitate extraction (%)	39.4 ± 3.3	24.0 ± 4.7[d]	42.8 ± 3.8	43.1 ± 4.1
^{3}H-oleate extraction (%)	41.7 ± 2.2	29.9 ± 5.6	42.0 ± 2.6	43.6 ± 4.4
FFA uptake (μeq/min)	2.45 ± 0.89	5.21 ± 2.15	2.11 ± 0.58	7.50 ± 2.0
FFA oxidation (μeq/min)	0.88 ± 0.32	3.10 ± 1.06[d]	0.25 ± 0.10	4.86 ± 1.58[e]
FFA release (μeq/min)	3.85 ± 1.25	2.03 ± 0.76	3.57 ± 0.98	7.17 ± 2.19
CO_2 prod. derived from FFA oxidation (%)	34.8 ± 8.5	24.9 ± 5.9	19.3 ± 4.2	32.6 ± 2.9[f]
FFA flux (μeq/min)	226 ± 26	232 ± 37	222 ± 29	320 ± 52
Art. glucose (μmoles/ml)	5.27 ± 0.17	5.28 ± 0.23	5.28 ± 0.25	6.43 ± 0.45[f]
Glucose uptake (μmoles/min)	9.59 ± 4.49	5.38 ± 7.20	10.78 ± 2.59	11.99 ± 4.43
Art. lactate (μmoles/ml)	0.83 ± 0.18	1.03 ± 0.26	2.41 ± 1.08	2.63 ± 0.87
Lactate output (μmoles/min)	4.47 ± 1.87	19.20 ± 8.64	5.19 ± 1.43	6.75 ± 4.31
Hematocrit (%)	46.0 ± 2.9	44.6 ± 2.8	44.3 ± 2.6	43.6 ± 1.6

[a] Mean ± SEM.

[b] Different from value at rest: [c] $p < 0.01$; [d] $p < 0.05$; different from value of recovery: [e] $p < 0.01$; [f] $p < 0.05$.

take) increased. Thus, during stimulation, 81% of the removed FFA was oxidized, while during rest, only 24% underwent oxidation. During recovery, this value dropped to about 7%. FFA oxidation could account for about 60% of the CO_2 production during rest. This value was not significantly influenced by muscle stimulation. Lactate output increased during stimulation. β-hydroxybutyrate was removed by the area during rest and showed

TABLE II. Changes in Skeletal Muscle Metabolism during Electrical Stimulation and Recovery in Diabetic Dogs ($N = 5$).

	Rest	Stimulation	Recovery
Blood flow (ml/min)	40.6 ± 3.3[a]	83.5 ± 9.0[b,c]	29.3 ± 3.5
Art. FFA (μeq/liter)	1545 ±182	1489 ±263	1526 ±266
^{14}C-palmitate extraction (%)	27.5 ± 2.8	16.9 ± 3.0[d]	24.8 ± 4.3
^{3}H-oleate extraction (%)	29.5 ± 2.8	21.5 ± 3.0	33.4 ± 4.9
FFA uptake (μeq/min)	10.6 ± 1.6	10.7 ± 2.8	6.6 ± 1.1
FFA oxidation (μeq/min)	2.57 ± 0.73	8.65 ± 1.82[c]	0.49 ± 0.42
FFA release (μeq/min)	12.5 ± 2.0	7.6 ± 1.9	10.2 ± 3.3
CO_2 prod. derived from FFA oxidation (%)	56.9 ± 20.4	78.6 ± 13.4	33.8 ± 12.6
FFA flux (μeq/min)	972 ±185	722 ±176	723 ±165
Art. glucose (μmoles/ml)	24.81 ± 2.89	24.81 ± 3.94	24.26 ± 4.16
Glucose uptake (μmoles/min)	11.97 ± 23.15	33.20 ± 16.00	26.42 ± 5.93
Art. lactate (μmoles/ml)	0.447 ± 0.63	0.557 ± 0.106	0.592 ± 0.099
Lactate output (μmoles/min)	9.22 ± 1.78	23.95 ± 7.17[c]	14.46 ± 6.02
Art. glycerol (μmoles/ml)	0.053 ± 0.006	0.082 ± 0.016	0.074 ± 0.012
Glycerol output (μmoles/min)	0.976 ± 0.276	0.835 ± 0.488	0.605 ± 0.267
Art. β-hydroxybutyrate (μmoles/ml)	0.265 ± 0.059	0.255 ± 0.076	0.253 ± 0.090
β-hydroxybutyrate uptake (μmoles/min)	3.04 ± 1.05	6.67 ± 3.24	2.29 ± 0.94
Hematocrit (%)	43.2 ± 1.2	43.6 ± 1.1	42.8 ± 1.9

[a] Mean ± SEM.

[b] Different from value at rest: [c] $p < 0.01$; [d] $p < 0.05$.

no significant change during stimulation.

Discussion. Under the conditions of the present experiments, resting skeletal muscle removed about 40% of the labeled arterial palmitic or oleic acid. Thirty-six percent of the removed FFA was oxidized accounting for approximately 35% of the CO_2 production. Removal of glucose and output of lactate were also demonstrated during rest. No information is available from these data concerning the possible oxidation of other substrates (e.g., muscle glycogen, esterified plasma, and tissue lipids, etc.).

During electrical stimulation of the skeletal muscle, the oxidation of FFA increased and no glucose removal was found. The high output of lactate might indicate increased breakdown of muscle glycogen, as demonstrated by Hultman and Bergstrom (16) in man. The present findings in dogs further resembled human exercise with respect to FFA supplying 25% of the CO_2 carbon, a value very similar to that found by Havel *et al.* (7). These findings are also similar to the data of Frey (17) who found that in working muscle, "uptake of FFA fell considerably short of covering the oxygen consumption."

In the recovery period following stimulation, FFA oxidation fell to very low levels, thus FFA uptake far exceeded oxidation. This finding is compatible with replenishment of lipid stores in the muscle as suggested previously (7). Glucose uptake and lactate output were similar to the rates found during rest.

Administration of DNP increased blood flow and FFA uptake. Sixty-five percent of the removed FFA was oxidized. The uptake of glucose and the release of lactate were not altered.

During all phases of the experiment, a release of FFA was also found in addition to the uptake of this metabolite. Such a release was noted previously by several laboratories both in dog (8, 17) and in man (7, 18–20).

Both uptake and oxidation of FFA were elevated in diabetic dogs. Glucose uptake was quite variable as indicated by the large standard error. A significant uptake of β-hydroxybutyrate took place during rest, which, if completely oxidized, may account for about 23% of the CO_2 output. Thus, more than 80% of the CO_2 production may be derived from FFA and ketone oxidation in the diabetic muscle. The release of FFA was considerably greater than in control dogs. During stimulation of the muscle, FFA oxidation increased, and FFA plus β-hydroxybutyrate oxidation may have accounted for all the CO_2 production.

During recovery, only a small fraction of the removed FFA was oxidized; a finding which is similar in control and diabetic animals. Glucose uptake was more consistent during this phase of the experiment. Oxidation of the removed β-hydroxybutyrate could have yielded about 20% of the CO_2 output.

Summary. The uptake and oxidation of FFA by skeletal muscle were studied in anesthetized, control dogs during rest, electrical stimulation, recovery, and DNP administration. Similar studies were also conducted in diabetic animals during rest, stimulation, and recovery. During rest, skeletal muscle removed about 40% of the labeled FFA and oxidized 36% of the removed amount. During skeletal muscle stimulation, the oxidation of FFA increased. During the recovery period, FFA oxidation was very low and accounted for about 12% of the uptake. The DNP also increased FFA oxidation. Both the uptake and oxidation of FFA by skeletal muscle were elevated in diabetic dogs and a significant uptake of β-hydroxybutyrate was also noted. A further increase of FFA oxidation occurred during stimulation. During recovery, only 7% of the removed FFA was oxidized and glucose uptake was high.

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The Effects of D-Penicillamine on Taste Preference and Volume Intake of Sodium Chloride by the Rat (33925)

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Studies in man have demonstrated that D-penicillamine produces hypogeusia, a decrease in taste acuity, in 20–40% of the patients treated with this drug (1, 2). This decrease in taste acuity affected each of the four qualities of taste but was most noticeable subjectively for salt and sweet stimuli. The present experiments were carried out to determine the effect of D-penicillamine on preference behavior and fluid intake for salt solutions in the rat.

Methods. The studies were carried out in two parts, an initial experiment (A) and a final experiment (B). (A) In the initial experiment 8 weanling Holtzman male rats, 21 days of age, were individually caged. Four rats served as controls and were fed a diet of ground Purina chow diluted with 15% added dextrose to increase palatability. This diet contained adequate calories, vitamins and minerals necessary for normal rat growth. Four rats served as experimental animals and received the control diet to which D-penicillamine[2] was added at a rate of 1 g/100 g of mixed feed. Intake of food and distilled water, both of which were supplied *ad libitum*, was recorded daily and animal weights were recorded every third day. Daily weights were interpolated from these values by assuming a linear relationship of the weight change over each 3-day period. Nineteen days after the diets were begun, each rat was offered a choice between 0.15 *M* sodium chloride (NaCl), reagent grade, and water under standard conditions for preference testing, in a two-bottle-choice experiment (3). With these techniques evaporation and waste were negligible. For the purpose of the studies this day was considered day 0. Thirty-one days after the diets were begun (day 12 of the study), the experimental rats were given the control diet without added D-penicillamine and this diet was continued until the termination of Expt. A. Intake of NaCl solution was recorded daily and expressed as percentage preference; *i.e.*, (ml of NaCl solution

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[2] D-penicillamine (Cuprimine) was kindly supplied as the free base by Merck, Sharpe and Dohme from Dr. Elmer Alpert.