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Glucose Metabolism in Perfused Skeletal Muscle

EFFECTS OF STARVATION, DIABETES, FATTY ACIDS, ACETOACETATE, INSULIN AND EXERCISE ON GLUCOSE UPTAKE AND DISPOSITION

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1. The regulation of glucose uptake and disposition in skeletal muscle was studied in the isolated perfused rat hindquarter. 2. Insulin and exercise, induced by sciatic-nerve stimulation, enhanced glucose uptake about tenfold in fed and starved rats, but were without effect in rats with diabetic ketoacidosis. 3. At rest, the oxidation of lactate (0.44 μmol/min per 30 g of muscle in fed rats) was decreased by 75% in both starved and diabetic rats, whereas the release of alanine and lactate (0.41 and 1.35 μmol/min per 30 g respectively in the fed state) was increased. Glycolysis, defined as the sum of lactate+alanine release and lactate oxidation, was not decreased in either starvation or diabetes. 4. In all groups, exercise tripled O_2 consumption (from ~8 to ~25 μmol/min per 30 g of muscle) and increased the release and oxidation of lactate five- to ten-fold. The differences in lactate release between fed, starved and diabetic rats observed at rest were no longer apparent; however, lactate oxidation was still several times greater in the fed group. 5. Perfusion of the hindquarter of a fed rat with palmitate, octanoate or acetoacetate did not alter glucose uptake or lactate release in either resting or exercising muscle; however, lactate oxidation was significantly inhibited by acetoacetate, which also increased the intracellular concentration of acetyl-CoA. 6. The data suggest that neither glycolysis nor the capacity for glucose transport are inhibited in the perfused hindquarter during starvation or perfusion with fatty acids or ketone bodies. On the other hand, lactate oxidation is inhibited, suggesting diminished activity of pyruvate dehydrogenase. 7. Differences in the regulation of glucose metabolism in heart and skeletal muscle and the role of the glucose/fatty acid cycle in each tissue are discussed.

The present paper deals with the alterations in muscle-glucose metabolism that occur in starvation and diabetes and the role of free fatty acids and ketone bodies in causing these changes. Much of our present knowledge of this subject has been derived from experiments carried out in the perfused rat heart. Using this preparation, Randle *et al.* (1966) observed an inhibition of the uptake, phosphorylation and oxidation of glucose and a block in glycolysis at the level of phosphofructokinase both in starvation and diabetes. In addition, they observed a similar pattern in hearts perfused with fatty acids and ketone bodies, which led them to suggest that the increased utilization of these substrates in starved and diabetic rats might explain the impaired glucose metabolism in these states. Comparable findings have been observed in diaphragm by some laboratories but not others (see Ruderman *et al.*, 1969).

It has been widely assumed that a similar type of regulation occurs in the large mass of voluntary skeletal muscle; however, the available experimental data do not support this notion. In both adductor-muscle fibres of the rhesus monkey (Beatty & Bocek, 1971) and the isolated perfused rat hindquarter (Houghton & Ruderman, 1971; Jefferson, *et al.*, 1972; Goodman *et al.*, 1974; Reimer *et al.*, 1974), exogenous fatty acids and ketone bodies do not inhibit insulin-stimulated glucose uptake. Further, the characteristic changes in tissue glycogen, citrate and hexose monophosphates noted in hearts of starved and diabetic rats are not seen in skeletal muscle (Adrouny, 1969; Goodman *et al.*, 1974).

In earlier studies (see Goodman *et al.*, 1974; Berger *et al.*, 1975), we investigated the effects of starvation, diabetes, fatty acids, acetoacetate and insulin on glucose uptake in the perfused rat hindquarter. In the present paper, the effects of these variables on glycogen formation, glycolysis, glucose oxidation and the concentration of metabolic intermediates in perfused muscle are described. In addition, as heart differs from voluntary skeletal

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muscle in that it is constantly contracting, we have carried out experiments during exercise as well as rest. A preliminary report of this work has already appeared (Berger *et al.*, 1974).

Materials and Methods

Animals

Female Sprague–Dawley rats weighing 180–250g were used. Rats were fed *ad libitum* on Purina Chow for at least 3 days or were starved for 48h before use. Diabetes was induced by an intravenous injection of streptozotocin (125mg/kg body wt.); the animals were allowed free access to food and water and were studied 72h after injection, by which time they were in severe diabetic ketoacidosis (Berger *et al.*, 1975). All procedures were carried out in rats anaesthetized with pentobarbital (3.5–5mg/100g body wt.) administered intraperitoneally.

Materials

Bovine serum albumin (Cohn fraction V, containing 0.8mg of fatty acid/g of albumin), obtained from Pentex Corp., Kankakee, IL, U.S.A., was dissolved in Krebs–Henseleit (1932) saline and dialysed twice against the bicarbonate saline as described previously (Ruderman & Goodman, 1973) for 24h. Palmitic acid, ethyl acetoacetate and octanoate were obtained from Sigma Chemical Co., St. Louis, MO, U.S.A.; L-[1-^{14}C]lactate was from New England Nuclear Corp., Boston, MA, U.S.A.; all enzymes and substrates for metabolic assays were from Boehringer Mannheim Corp., New York, NY, U.S.A. Acetoacetate, free of ethanol, was prepared from its ethyl ester as described by Krebs & Eggleston (1941). Free fatty acids, as their sodium salts, were added to the albumin before dialysis.

Perfusion procedure

Details of the perfusion apparatus, the operative preparation of the hindquarter and the composition of the perfusate were described previously (Ruderman *et al.*, 1971). The operative procedure differed slightly in that the skin of both hindlimbs was only partially removed to allow freeze-clamping of the muscle at the end of an experiment.

After placement in the perfusion apparatus, the hindquarter was cyclically perfused with 150ml of the standard medium, which contained 7.5mM-glucose, 4g of albumin/100ml, 1.5–2mM-lactate, 0.15mM-pyruvate and 3–5μCi of [1-^{14}C]lactate. The flow rate through the preparation (11ml/min) was monitored with a Gilmont flow meter (A. S. Thomas Corp., Philadelphia, PA, U.S.A.). Throughout the perfusion, pressure in the tubing leading to the aortic catheter was 8.0–9.5kPa (60–70mmHg).

Perfusion was carried out for 10min to allow equilibration, after which zero-time samples were collected. The hindquarter was then perfused for 15min in the resting state and for a second 15min during which time the muscle was contracting. To stimulate the muscle, Dastre's electrodes were attached to the sciatic nerves as described previously (Ruderman *et al.*, 1971). The legs were fixed on the perfusion platform with adhesive tape and isometric contractions were induced with a Grass SK2 stimulator. Electrical stimuli were applied for 0.1ms at a frequency of 5/s. The voltage at the beginning of the exercise period was 0.1–0.5V; however, to obtain vigorous contractions for 15min, considerable increases in the voltage, up to as much as 5V, were sometimes necessary, towards the end of the perfusion period. With this method of stimulation, approximately one-third of the muscle of the hindquarter is intensely contracting (Houghton, 1971).

Exercise caused only minor changes in the flow of perfusate through the preparation, and by adjusting the pressure in the arterial portion of the circulation, the flow rate was kept constant at 11ml/min. Throughout the perfusion, the mean pressure in the tubing leading to the aortic catheter was 8.0–9.5kPa (60–70mmHg), the same as in the experiments with resting muscle.

Sampling of perfusate and tissue

Duplicate samples of perfusate were taken after 0, 15 and 30min, and production and utilization rates were calculated from changes in perfusate concentration. Comparisons of resting and exercising muscle were based on values obtained in the first and second 15min of perfusion. Metabolic activity of the resting hindquarter is constant for at least 30–60min (Ruderman *et al.*, 1971); therefore differences between the exercise and resting periods cannot be attributed to metabolic alterations related to the duration of perfusion.

Tissue metabolites and lactate specific radioactivity were determined in muscle freeze-clamped (see Ruderman *et al.*, 1971) at the end of the experiment. To obtain samples from resting muscle, a separate set of experiments was carried out in which the perfusion was terminated at 15min. The radioactivity of resting muscle did not change significantly during a 15min perfusion: 45000$\pm$14000d.p.m./g (mean$\pm$S.D.; $n = 6$) at zero time and 53200$\pm$6400d.p.m./g at 15min. In addition, the specific activity of lactate, which accounted for more than 90% of the total radioactivity (see below), was very comparable in perfusate and tissue, making it possible to calculate the mean specific radioactivity of lactate in resting muscle from perfusate values. This was done only in experiments with resting muscle. In these studies, the specific radioactivity of

lactate in tissue was determined from the mean of lactate specific radioactivity in the perfusate at 0 and 15min. This both enabled us to carry out exercise studies in the same hindquarter and to correct for changing lactate specific radioactivity during the experimental period.

In exercising muscle the specific radioactivity of lactate in muscle-cell water was determined from whole-tissue measurements after correcting, by the following expression for contamination with extracellular fluid (see Berger *et al.*, 1975).

$$\text{Specific activity of lactate in muscle cells (as d.p.m./}\mu\text{mol of lactate)} = \frac{a-0.2b}{A-0.2B}$$

where a is the amount of radioactivity in muscle as d.p.m./g of wet muscle; b is the amount of radioactivity in perfusate water as d.p.m./ml; A is the muscle content of lactate as μmol/g of wet muscle; B is the concentration of lactate in perfusate water as mM.

It was assumed that extracellular fluid comprises 20% of muscle mass (see Berger *et al.*, 1975) and that nearly all the radioactivity in perfusate and tissue was due to lactate (see below).

In agreement with the findings of others (Sacktor *et al.*, 1965; Corsi *et al.*, 1969), vigorous isometric exercise caused the concentration of lactate in muscle to increase to approx. 12μmol/g within 2min and it remained at this value for the duration of the perfusion (Fig. 1). Since tissue radioactivity remained constant [51000±3500d.p.m./g (mean±S.D.; $n=6$)], the specific radioactivity of lactate in muscle-cell water at 30min was taken to be representative of the entire 15min period. Perfusate radioactivity also remained constant during the exercise period. Correction was not made for the increase in perfusate lactate (from 2–2.5 to 4–4.5mM) as this would have had a minimal effect (see eqn. 1).

Isotopic methods

The method for collecting $^{14}CO_2$ has been described previously (Ruderman *et al.*, 1971). Serial sampling of perfusate and tissue extracts revealed that isotopic equilibrium between the two was achieved at the end of the equilibration period and persisted throughout the entire perfusion. T.l.c. of $HClO_4$ extracts of medium or tissue homogenates on silica gel [solvent systems: ether/sorbic acid/water (7:2:1 by vol.) or propanol/aq. NH_3 (7:3 v/v)] established that more than 90% of the radioactivity could be recovered from a single spot with the R_F value of lactate, even at the end of an experiment. Therefore radioactivity in other compounds was neglected, and specific radioactivity of lactate was determined in perfusate and tissue by dividing the total d.p.m./volume by the lactate concentration.

The oxidation of lactate provides an indirect index of glucose oxidation, since lactate is constantly formed from glucose during the course of a perfusion, and very little glucose is oxidized by the pentose shunt in skeletal muscle (Green & Landau, 1965). [1-^{14}C]-Lactate was used in the present study, in place of radioactive glucose, to limit difficulties in quantification caused by the dual origin of glycolytic intermediates from exogenous glucose and glycogen. In separate experiments, we established that in resting muscle, lactate oxidation was linear during the two 15min experimental periods after equilibration.

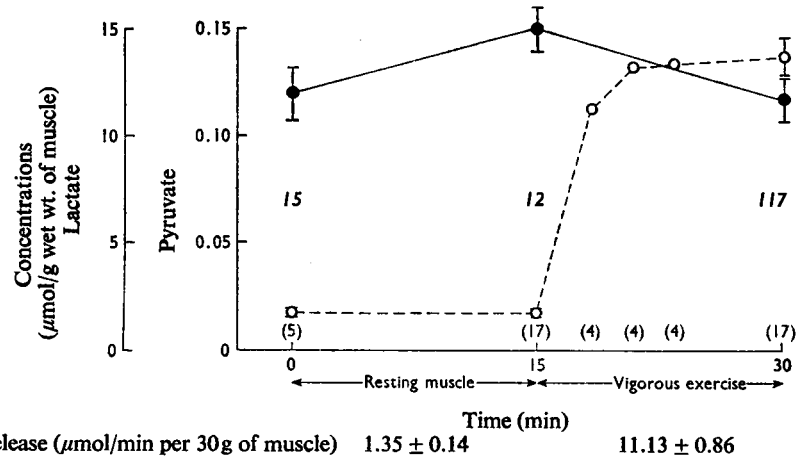
Analytical methods

Perfusate and tissue specimens (after powdering under liquid N_2) were deproteinized in ice-cold 6% (w/v) $HClO_4$. The extracts were neutralized with KOH, and the $KClO_4$ precipitate was removed by centrifugation at ~2000g for 7–10min. Acetylcarnitine was assayed fluorimetrically by the method of Williamson & Corkey (1969). The methods used for other metabolite assays and for O_2 determinations were described previously (Goodman *et al.*, 1974; Berger *et al.*, 1975; Ruderman & Berger, 1974).

Calculations

Uptake and release of metabolites were determined from changes in their concentration in the perfusate and are expressed as μmol/min per 30g of muscle (see Ruderman *et al.*, 1971). Acetoacetate utilization was corrected for non-enzymic losses and for acetoacetate converted into 3-hydroxybutyrate (Ruderman & Goodman, 1973). Lactate oxidation was determined from the difference in total $^{14}CO_2$ generated at the beginning and the end of an experimental period and was corrected for specific radioactivity of lactate as described above.

Flux rates through metabolic pathways were calculated as described below by using mean values for glucose uptake, lactate release, lactate oxidation, alanine release and, in the exercise studies, the accumulation of lactate in the contracting leg muscles. The contribution of pyruvate accumulation to glycolysis was not determined; however, in other experiments we have established that pyruvate accumulation in perfusate and tissue is negligible both at rest (approx. 10% that of lactate; results not shown) and during exercise (see Table 3). (1) Lactate production = lactate release + lactate accumulation in the tissue. During perfusions with the hindquarter at rest, there was no accumulation of lactate within the muscle tissue of the legs (Fig. 1); it was assumed that during exercise lactate accumulated only in the intensely contracting leg muscles, i.e. in about one-third of the hindquarter muscle mass. (2) Glycolytic flux = (lactate production + lactate oxidation + alanine release)/2. Since most of the carbon of alanine is



Lactate release (μmol/min per 30g of muscle) 1.35 ± 0.14 11.13 ± 0.86

Fig. 1. *Concentrations of lactate and pyruvate in perfused hindquarters of fed rats at rest and during exercise*

Results are means ± S.E.M., with the number of observations at each time-point in parentheses. The initial tissue-lactate value during vigorous exercise was taken at 17 min. Lactate release was determined from differences in perfusate concentrations over a 15 min interval. See the Materials and Methods section for further details. ○, Lactate; ●, pyruvate. [Lactate]/[pyruvate] values are shown in italics.

thought to be derived from glucose (see Odessey *et al.*, 1974), the contribution of proteolysis was neglected. (3) Net glycogen synthesis = glucose uptake − glycolytic flux. Negative values indicate net glycogen breakdown.

Results

Fed rats

At rest, the uptake of glucose by the hindquarter of a fed rat was negligible when insulin was not added to the perfusate (Table 1). On the other hand, lactate and alanine, which are thought to be principally derived from glucose (see Felig, 1973; Odessey *et al.*, 1974), were released at rates of 1.35 and 0.41 μmol/min per 30g of muscle respectively, and lactate was oxidized at a rate of 0.44 μmol/min per 30g of muscle. As a result, the magnitude of glycolysis was substantially greater than that of glucose uptake, suggesting that much of the lactate and alanine released by the hindquarter was derived from muscle glycogen.

As shown previously, insulin markedly enhances the uptake of glucose in resting muscle of fed rats, but it does not significantly affect the release of either lactate or alanine (see Ruderman *et al.*, 1971; Ruderman & Berger, 1974). Insulin significantly enhanced lactate oxidation; however, the overall rate of glycolysis was only slightly increased, indicating that the majority of the glucose taken up by the insulin-treated muscle is deposited as glycogen.

Vigorous isometric exercise caused a threefold increase in O_2 consumption (Table 2), a marked decrease in the tissue concentrations of creatine phosphate and ATP, and increases in muscle lactate and the lactate/pyruvate ratio (Fig. 1, Table 3). This is in agreement with earlier findings reported for exhaustive exercise in man (Diamant *et al.*, 1968; Knuttgen & Saltin, 1972; Karlsson & Ollander, 1972) and experimental animals (Corsi *et al.*, 1969; Hirche *et al.*, 1973). In the absence of added insulin, exercise increased the uptake of glucose, glycolysis, glycogen breakdown and the oxidation of lactate approximately tenfold. By contrast, the release of alanine was, if anything, diminished. The addition to the perfusate of insulin (10m-i.u./ml) did not alter either the decrease in muscle creatine phosphate and ATP (see Berger *et al.*, 1975) or the increases in lactate release and oxidation and glycolysis caused by exercise (Table 1), although it did significantly diminish the release of alanine. Insulin also increased glucose uptake and diminished net glycogenolysis in the exercising hindquarter; however, the interpretation of these findings is difficult, as only approximately one-third of the muscle of the hindquarter is intensely contracting during sciatic-nerve stimulation (Houghton, 1971). Thus it remains to be determined whether insulin specifically stimulates the uptake of glucose and/or diminishes glycogen breakdown in the contracting tissue.

As shown in Table 2, the oxidation of lactate accounted for at least 17% of the oxidative metabolism of the hindquarter of a fed rat at rest and at

Table 1. *Glucose uptake and disposition in perfused hindquarters of fed, starved and diabetic rats*

Results are means ± S.E.M. with numbers of observations in parentheses. Insulin (10m-i.u./ml) was added to initial perfusate as indicated. Exercise was produced by sciatic-nerve stimulation at a rate of 5/s. Negative values for glycogen synthesis indicate net glycogenolysis. Values for resting and exercising muscle were obtained during the first and second 15min of perfusion respectively. See the Materials and Methods section for further details.

Experimental conditions	Insulin	Release (μmol/min per 30g of muscle) of: Glucose uptake	Lactate	Alanine	Lactate oxidation	Calculated rate of glycolysis (μmol of glucose/min per 30g of muscle)	Calculated net glycogen synthesis (μmol of glucose/min per 30g of muscle)
Fed							
Rest	−	0.49 ± 0.24 (34)	1.35 ± 0.14 (21)	0.41 ± 0.02 (19)	0.44 ± 0.04 (17)	1.1	−0.6
Rest	+	5.39 ± 0.48 (14)*	1.52 ± 0.27 (14)	0.37 ± 0.03 (18)	0.71 ± 0.08 (14)*	1.3	4.0
Exercise	−	4.01 ± 0.34 (17)†	11.13 ± 0.86 (17)†	0.32 ± 0.03 (14)†	4.91 ± 0.89 (9)†	12.4	−8.4
Exercise	+	8.41 ± 0.63 (10)*†	11.42 ± 0.77 (10)†	0.21 ± 0.03 (14)*†	5.66 ± 0.57 (5)†	11.9	−3.5
Starved, 48h							
Rest	−	0.30 ± 0.29 (8)	2.21 ± 0.27 (8)‡	0.57 ± 0.06 (8)§	0.12 ± 0.02 (9)‡	1.5	−1.2
Rest	+	5.30 ± 0.79 (6)†	1.89 ± 0.39 (6)	0.44 ± 0.04 (6)	0.13 ± 0.02 (6)‡	1.2	4.1
Exercise	−	3.37 ± 0.49 (6)†	10.54 ± 0.87 (5)†	0.40 ± 0.07 (6)	1.73 ± 0.38 (6)†‡	9.5	−6.1
Exercise	+	8.57 ± 0.57 (6)*†	9.60 ± 1.11 (6)†	0.34 ± 0.05 (6)§	1.75 ± 0.36 (6)†‡	9.4	−0.8
Diabetic							
Rest	−	−0.52 ± 0.27 (10)‡	2.81 ± 0.56 (10)§	0.81 ± 0.05 (10)‡	0.11 ± 0.01 (9)‡	1.9	−2.4
Rest	+	2.02 ± 1.14 (10)‡	2.61 ± 0.44 (10)§	0.56 ± 0.04 (10)*‡	0.17 ± 0.03 (9)‡	1.7	0.4
Exercise	−	0.68 ± 0.50 (10)‡	12.82 ± 1.53 (10)†	0.90 ± 0.09 (9)‡	1.09 ± 0.13 (9)†‡	10.4	11.1
Exercise	+	6.07 ± 1.13 (9)*†	15.46 ± 1.69 (9)†	0.54 ± 0.05 (10)*‡	2.28 ± 0.33 (9)*†‡	11.92	−5.8

* Value significantly different from that of hindquarter perfused without added insulin, $P < 0.01$
† Value different from that of resting muscle, $P < 0.01$
‡ Value different from that of comparable hindquarter of fed group, $P < 0.01$
§ As for ‡, $P < 0.05$

Table 2. *Contribution of lactate to the oxidative metabolism of the isolated perfused rat hindquarter: effect of starvation, diabetes and exercise*

Results are means±S.E.M. with the number of observations in parentheses. Insulin was not added to the perfusate. As explained in the text, $^{14}CO_2$ production from [1-^{14}C]lactate provides a minimum estimate of the rate of glucose oxidation. See the Materials and Methods section and the legend to Table 1 for further details.

Experimental conditions	O_2 consumption (μmol/min per 30g of muscle)	Lactate oxidation	Percentage of O_2 used accounted for by lactate oxidation*
Fed			
Rest	7.6±0.5 (4)	0.44±0.04 (17)	17
Exercise	23.4±2.4 (5)	4.91±0.89 (9)	63
Starved, 48h			
Rest	9.0±1.2 (8)	0.12±0.02 (9)	4
Exercise	20.7±4.6 (3)	1.73±0.38 (6)	25
Diabetic			
Rest	9.6±0.8 (4)	0.11±0.01 (9)	3
Exercise	23.4±2.2 (7)	1.09±0.13 (9)	14

* Assumes 3mol of O_2 used per mol of lactate oxidized.

Table 3. *Effects of free fatty acids and acetoacetate on tissue metabolite concentrations in perfused skeletal muscle of fed rats during rest and isometric exercise*

See the legend to Table 4 for other details.

Experimental conditions	Concentration (μmol/g wet weight)					
	Pyruvate	Lactate	ATP	Creatine phosphate	Fructose 6-phosphate	Citrate
Rest	0.15±0.01 (17)	1.69±0.17* (17)	4.91±0.14* (11)	14.67±0.48* (11)	0.06±0.01* (12)	0.24±0.02 (12)
Exercise	0.12±0.01 (17)	14.01±1.00 (19)	3.84±0.22 (11)	5.93±0.66 (11)	0.23±0.04 (10)	0.19±0.01 (11)
Exercise+palmitate (1.3mM)	0.16±0.02 (7)	10.80±2.09 (7)	4.42±0.48 (7)	6.23±0.91 (7)	0.28±0.03 (8)	0.20±0.01 (7)
Exercise+octanoate (1mM)	0.16±0.02 (5)	10.21±1.73 (5)	3.98±0.33 (5)	9.68±1.20 (5)	0.20±0.04 (4)	0.17±0.03 (4)
Exercise+acetoacetate (1.8mM)	0.15±0.01 (10)	12.49±1.46 (9)	3.77±0.30 (10)	5.50±1.18 (10)	0.28±0.04 (10)	0.27±0.03† (10)

* Same as for †, $P<0.01$.
† Significantly different from exercised muscle perfused with no additions, $P<0.05$.

least 63% during vigorous exercise. These are minimum estimates of lactate oxidation, since the specific radioactivity of pyruvate was not measured. Therefore if lactate and pyruvate were not in equilibrium, lactate oxidation would be underestimated. The fact that 63% of the O_2 used by the hindquarter during exercise could be accounted for by lactate oxidation suggests that such an error would be minor, since if significant isotopic disequilibrium occurred, it should be most apparent during exercise when the rate of pyruvate formation is greatly increased.

Starved and diabetic rats

In the absence of insulin, glucose uptake was not significantly different from 0 in resting muscle of starved and diabetic rats (Table 1). Compared with the fed group, the release of alanine and lactate were increased by 50–100% and lactate oxidation was decreased by 70%. The disparity between glucose uptake and glycolysis was greater than in the fed group, suggesting an increased rate of glycogenolysis. As reported previously, insulin enhanced the uptake of glucose in starved rats, but not in severely diabetic

rats (Goodman *et al.*, 1974; Berger *et al.*, 1975). Insulin did not alter the release or oxidation of lactate in either group. On the other hand, it substantially diminished the release of alanine in diabetic rats and marginally diminished it in starved rats.

Electrical stimulation of the sciatic nerves caused increments in O_2 consumption (Table 2) and decreases in tissue creatine phosphate and ATP very similar to those noted in fed rats (see Berger *et al.*, 1975), suggesting that muscle contractions were of comparable intensity. In the starved group, the overall response to exercise, both in the presence and absence of insulin, was qualitatively similar to that of fed rats except that insulin did not decrease alanine release; lactate oxidation was increased approximately 15-fold, but was still substantially less than in the fed group.

Exercise did not increase glucose uptake in severely diabetic rats unless insulin was added to the perfusate, as described previously (Berger *et al.*, 1975). On the other hand, it increased the release and oxidation of lactate and the overall rate of glycolysis as it did in the other groups. In contrast with starved rats, insulin enhanced the oxidation of lactate (cf. fed group), although the absolute rate of lactate oxidation was still low. It is noteworthy that glycolysis occurred at the same or a slightly higher rate in diabetic rats during exercise than in either of the other groups. Since glucose uptake is decreased in the diabetic rats, this suggests that the muscle of these animals relies more heavily on endogenous glycogen for its supply of glycolytic intermediates. The importance of this finding will be noted in the discussion.

The relative contribution of lactate (glucose) to the total oxidative metabolism of the hindquarter was by far the greatest in fed rats (Table 2). In these animals, lactate oxidation accounted for at least 17% of total O_2 uptake at rest and 63% of it during exercise. By contrast, after 48h of starvation lactate accounted for only 4% of the O_2 consumed at rest and 25% during exercise. In the diabetic group, the comparable values were 3 and 14%, although in the presence of insulin the contribution of lactate was undoubtedly more comparable with that of the starved rats because of the higher rate of lactate oxidation. Presumably, lactate (glucose) is replaced as a fuel in muscle of starved and diabetic rats by lipid and to some extent amino acids.

Effect of free fatty acids and ketone bodies

Inhibition of glucose uptake, glycolysis and pyruvate oxidation is observed in heart muscle of starved and diabetic rats and can be reproduced by perfusing the heart with fatty acids (see Randle *et al.*, 1966). To determine whether free fatty acids have a similar effect in voluntary skeletal muscle, hindquarters were perfused with media containing added palmitate or octanoate. The latter was used in case carnitine-mediated transport into the mitochondria was limiting for the oxidation of long-chain fatty acids. As shown in Table 4, neither fatty acid affected glucose uptake, lactate oxidation, the release of lactate and alanine or net glycolysis.

Acetoacetate has also been shown to inhibit glucose metabolism in perfused heart (Randle *et al.*, 1966). In addition, it is used in preference to fatty acid as a fuel by skeletal muscle (see Ruderman *et al.*, 1971). When added to the initial medium at a concentration of 1.8mM, acetoacetate was utilized at a rate of 2.41 ± 0.2 (mean $\pm$ S.E.M., $n = 10$)

Table 4. *Effects of free fatty acids and acetoacetate on glucose uptake and disposition in resting and exercising muscle of fed rats*

Results are means $\pm$ S.E.M. with the number of observations in parentheses. Fatty acids and acetoacetate were added to the initial medium as indicated. In addition, fatty acid (0.3mM) was present in all perfusates which was due to fatty acid already bound to the albumin. Insulin was not added to the perfusing medium. See the legend to Table 1 and the Materials and Methods section for further details.

Experimental conditions	Glucose uptake	Lactate release	Lactate oxidation	Alanine release	Calculated rate of glycolysis
		(μmol/min per 30g of muscle)			
Rest					
No additions	0.49 ± 0.24 (34)	1.35 ± 0.14 (21)	0.44 ± 0.04 (17)	0.41 ± 0.02 (19)	1.1
Palmitate (1.3mM)	0.77 ± 0.46 (7)	1.24 ± 0.21 (8)	0.68 ± 0.06 (5)	0.41 ± 0.03 (7)	1.2
Octanoate (1mM)	0.03 ± 0.38 (5)	0.83 ± 0.40 (5)	0.52 ± 0.05 (5)	0.46 ± 0.03 (4)	0.9
Acetoacetate (1.8mM)	0.40 ± 0.45 (10)	1.03 ± 0.20 (10)	0.35 ± 0.03 (9)*	0.39 ± 0.06 (9)	0.9
Exercise					
No additions	4.01 ± 0.34 (17)	11.13 ± 0.86 (17)	4.91 ± 0.89 (9)	0.32 ± 0.03 (19)	12.4
Palmitate (0.3mM)	2.95 ± 0.80 (7)	12.07 ± 1.40 (7)	4.41 ± 1.07 (7)	0.38 ± 0.03 (7)	11.4
Octanoate (1mM)	3.52 ± 0.61 (5)	8.41 ± 1.31 (5)	3.72 ± 0.36 (5)	0.38 ± 0.05 (4)	9.1
Acetoacetate (1.8mM)	3.25 ± 0.51 (10)	9.12 ± 1.00 (7)	2.84 ± 0.61 (5)*	0.41 ± 0.05 (8)	9.8

* Significantly different from controls, $P < 0.05$.

Table 5. *Effect of acetoacetate on tissue metabolites in isolated perfused skeletal muscle of rats starved for 48 h*

Results are means±S.E.M. with the number of observations in parentheses. Hindquarters were perfused with standard media (see Materials and Methods section) for 15 min without recirculation by the flow-through technique (see Ruderman & Goodman, 1973), at which time the muscle was freeze-clamped. Acetoacetate utilization was calculated from arteriovenous differences and flow rate and was corrected for 3-hydroxybutyrate formation.

Additions to perfusate	Acetoacetate utilized (μmol/min per 30g of muscle)	Tissue concentration (nmol/g wet weight)				
		Acetoacetate	Citrate	Acetyl-CoA	Free CoA	Acetylcarnitine
No additions (4)	—	91±9	237±35	1.75±0.39	6.7±0.65	36
Acetoacetate, 0.84mM (3)	1.44±31	144±26	286±12	5.20±0.90	5.3±0.44	102
Acetoacetate, 1.73mM (3)	2.11±56	470±71	264±46	5.20±1.2	6.2±0.53	133

Table 6. *Effect of acetoacetate in glucose utilization by the perfused rat hindquarter*

Results are means±S.E.M. for four experiments in each group. Hindquarters were not exercised. Fat-poor albumin (Miles Laboratories, Kankakee, IL, U.S.A.) was used, and initial free fatty acid concentration in perfusate was less than 0.1mM. Acetoacetate and insulin were added after 15min of perfusion, and glucose utilization was measured over the subsequent 45min. Concentration of acetoacetate at 60min was 1.4mM.

	Glucose utilization (μmol/min per 30g of muscle)
Control	0.70±0.14
Insulin (0.2m-i.u./ml)	4.58±0.37
Insulin+acetoacetate (2mM)	4.25±0.66

μmol/min per 30g of muscle. The uptake of glucose and the release of lactate and alanine were unaffected; however, the oxidation of lactate was significantly inhibited (Table 4). Determination of tissue metabolites in hindquarters perfused with 0.84 or 1.73mM-acetoacetate revealed threefold increases in acetylcarnitine and acetyl-CoA (Table 5). The concentration of free CoA was unchanged; however, the increase in the acetyl-CoA/CoA ratio provides a possible basis for the inhibition of lactate oxidation (see the Discussion section). Muscle citrate was not increased. As shown in Table 6, acetoacetate also did not affect glucose utilization when the hindquarter was perfused with a physiological concentration of insulin and a fatty acid-poor albumin.

Perfused heart differs from voluntary skeletal muscle in that it is constantly contracting, and glucose accounts for a sizeable portion of its fuel needs, even in the absence of added insulin. A similar situation occurs in voluntary muscle during exercise. Therefore we investigated the effects of octanoate, palmitate and acetoacetate on glucose metabolism of fed rats during isometric exercise. As shown in Table 3, vigorous exercise caused similar changes in muscle lactate, creatine phosphate and ATP in all groups, suggesting that comparable degrees of exercise were performed. None of the agents altered the stimulatory effect of exercise on glucose uptake or lactate release (Table 4), and neither palmitate nor octanoate significantly inhibited lactate oxidation, although small effects might have been missed due to the great variability of this measurement. By contrast, acetoacetate inhibited the oxidation of lactate by nearly 50%. There was a borderline increase in citrate in the hindquarters perfused with acetoacetate, but no change occurred as a result of exposure to octanoate or palmitate. Glycolysis was somewhat diminished in the hindquarters perfused with acetoacetate and octanoate; however, the significance of this finding is not clear, since statistical analysis was not possible. That it does not reflect inhibition of phosphofructokinase is suggested by the observation that fructose 6-phosphate was increased to a similar extent by exercise in all groups.

Discussion

Starvation

During starvation of more than 12–24h, the oxidation of glucose in muscle (Andres *et al.*, 1956) and other tissues in man is decreased to conserve glucose for the brain (Cahill & Owen, 1968). As pointed out by Cahill & Owen (1968) and Cahill (1970), if this did not occur gluconeogenesis would have to be maintained at a very high rate and protein wasting would be excessive. The present results suggest that inhibition of pyruvate oxidation in skeletal muscle is an important mechanism for achieving these ends. Thus even though rates of glycolysis are comparable in hindquarters of fed and starved rats, the oxidation of glucose (lactate) is only 25% as great in the starved group, and the release of

lactate and alanine is increased. Further, although glucose oxidation increases more than tenfold in starved rats during exercise, it is still far less than in fed animals, indicating that pyruvate oxidation is still relatively inhibited.

Our data may, if anything, underestimate the difference in lactate oxidation between fed and starved rats. Lactate and pyruvate are released by the hindquarter of an intact rat starved for 48h at approximately the same rate as in the isolated perfused preparation (Ruderman *et al.*, 1971). On the other hand, in fed rats lactate and pyruvate are taken up by the hindquarter *in vivo*, and if oxidized completely could account for approx. 40% of the O_2 consumed (MacDonald *et al.*, 1976). The reason for this difference has not been ascertained; nevertheless, the findings in the intact rat suggest that glucose and lactate account for an even greater percentage of the oxidative substrate of muscle in the fed state *in vivo* than our results indicate.

The basis for the inhibition of pyruvate oxidation in skeletal muscle during starvation is only partially understood. The activity of the active dephosphorylated form of pyruvate dehydrogenase is diminished in skeletal muscle (Hagg *et al.*, 1975, 1976) as it is in heart (Wieland *et al.*, 1971), liver (Wieland *et al.*, 1974) and adipose tissue (Stansbie *et al.*, 1975) during starvation, and the concentration of acetyl-CoA (an inhibitor of the active form of pyruvate dehydrogenase) is increased (Goodman *et al.*, 1974). Studies carried out with the perfused rat heart suggest that such changes could be the result of an increased availability of ketone bodies and/or fatty acids. In accordance with this, we have been able to demonstrate inhibition of glucose oxidation associated with an increase in tissue acetyl-CoA (the present study) and a decrease in the activity of the active form of pyruvate dehydrogenase (Hagg *et al.*, 1975, 1976) by perfusing the hindquarter with acetoacetate. On the other hand, we have been unable to inhibit oxidation (acetyl-CoA and the active form of pyruvate dehydrogenase were not measured) by perfusing with a high concentration of palmitate or octanoate. This contrasts with the finding of Beatty & Bocek (1971) that incubation with palmitate causes a 30% inhibition of glucose oxidation in sartorius-muscle fibres of the rhesus monkey. The reason for these differing results remains to be established.

In vivo, the uptake of glucose by muscle is diminished during starvation, at least in part, due to the decreased ambient concentrations of both glucose and insulin (see Berger *et al.*, 1975). That there is probably no specific alteration of the glucose-transport mechanism is suggested by the fact that rates of glucose uptake in hindquarters of fed and starved rats are comparable both at rest, when perfused with the same concentrations of insulin and glucose, and during exercise (Table 1).

Effect of diabetes

As noted previously, the stimulation of glucose transport by both exercise and insulin is decreased in hindquarters of severely diabetic rats (Berger *et al.*, 1975). The present data demonstrate that diabetic rats release somewhat more lactate and alanine than do starved rats, but that the rate of lactate oxidation, both at rest and during exercise, is comparable in the two groups. They also reveal that the diabetic rat is more dependent on muscle glycogen as a source of carbon atoms for glycolysis (Table 1). This was evident in resting muscle, but was most striking during exercise (no insulin), when glycolysis was markedly stimulated in both starved and diabetic rats, but the uptake of glucose was enhanced only in the starved group. Bergstrom & Hultman (1967) have demonstrated that exhaustion of muscle during severe exercise correlates with depletion of its glycogen stores. Whether the muscle of a severely diabetic subject is more prone to fatigue during exercise as a result of its greater dependence on endogenous glycogen remains to be determined.

Comparison of glucose uptake in heart and skeletal muscle

Glucose uptake. A comparison of data obtained with the perfused heart and hindquarter indicate that there are significant differences in the regulation of glucose metabolism in the two types of muscle (see Table 6). In the absence of insulin, the uptake of glucose by the resting hindquarter is negligible and there are no differences between fed, starved and diabetic rats (see Table 1). By contrast, the uptake of glucose by heart is significant, and it is diminished by both starvation and diabetes (Randle *et al.*, 1966; Ruderman *et al.*, 1976). Further, although the stimulation of glucose uptake by insulin is decreased in both tissues by diabetes, free fatty acids and ketone bodies cause a similar inhibition only in heart. Finally, the stimulatory effect of exercise on the uptake of glucose is not diminished by fatty acids or ketone bodies in the hindquarter, whereas the fatty acids markedly decrease the increase in cardiac glucose utilization caused by an increment in intraventricular pressure (Neely *et al.*, 1969).

The inability of fatty acids and ketone bodies to inhibit glucose uptake by the perfused hindquarter during maximal stimulation by insulin has previously been noted by ourselves and by Jefferson *et al.* (1972). In addition, Short *et al.* (1969) and Beatty & Bocek (1971) have reported that free fatty acids do not inhibit the stimulation of glucose uptake by insulin in isolated muscle fibres of man and monkey respectively. Supraphysiological concentrations of insulin were used in these as well as in the present study; however, we have observed similar results when

Table 7. *Glucose uptake and disposition in perfused rat heart and hindquarter*

Results are means of at least six experiments. Data for perfused heart are from Randle *et al.* (1966) and for hindquarter from this study and from Goodman *et al.* (1974).

State of rat	Free fatty acids	Insulin	Glucose uptake	Glucose oxidation (μmol glucose/h per g wet wt.)	Calculated rate of net glycolysis	Net glycogen synthesis
Resting hindquarter						
Fed	−	−	1	0.4	2.2	−1.2
Fed	+	−	1.5	0.7 (0.3)*	2.3	−1.2
Fed	−	+	11	0.7	2.6	8
Fed	+	+	14	—	—	—
Diabetic	−	+	4	0.1	2.8	1.2
Exercising hindquarter						
Fed	−	−	8	4.9	25	−17
Heart						
Fed	−	−	50	23	46	0
Fed	−	+	64	44	64	0
Fed	+	+	39	12	37	2
Diabetic	−	+	18	23	42	−24

* Perfusion with acetoacetate.

hindquarters are perfused with acetoacetate in the presence of as little as 200μ-i.u. of insulin/ml and a medium containing delipidated albumin (see Table 6).

Glucose disposition in heart and skeletal muscle

Heart and skeletal muscle also differ with respect to the disposition of glucose once it enters the cell (Table 7). This is most clearly seen by comparing the disposition of glucose taken up by the two tissues in the presence of insulin. In heart, nearly all the glucose is utilized for glycolysis: about 70% of it is oxidized and the remainder is released as lactate and pyruvate. By contrast, only 25% of the glucose taken up by the hindquarter can be accounted for by glycolysis and the remainder appears to be converted into glycogen. The finding that glucose disposition is more alike when one compares the exercising hindquarter with heart, suggests that some of these differences may be due to the fact that the heart is constantly contracting.

Another difference between heart and voluntary muscle relates to the regulation of glycolysis. Glycolysis in the two tissues is altered in the same way by such factors as contraction, alkalosis, anoxia and adrenaline (M. Berger, S. A. Hagg, M. N. Goodman & N. B. Ruderman, unpublished work). On the other hand, it is inhibited in heart but not in skeletal muscle by starvation, diabetes and perfusion with fatty acids or ketone bodies (cf. Randle *et al.*, 1966; Neely & Morgan, 1974; the present study). In accordance with these findings, we have not observed inhibition of phosphofructokinase, such as has been described in heart in these circumstances (Table 3) (Goodman *et al.*, 1974).

In contrast with glycolysis and glucose uptake, glucose (lactate) oxidation is inhibited equally in both heart and skeletal muscle by starvation and diabetes. The inhibition in both tissues is associated with increases in the ratio acetyl-CoA/CoA which could inhibit pyruvate dehydrogenase (see Randle *et al.*, 1966) and with a decrease in the fraction of the pyruvate dehydrogenase complex present in the active (dephosphorylated) form (Wieland *et al.*, 1971; Hagg *et al.*, 1975). Further, the entire picture can be reproduced by perfusion of the heart with fatty acids, and the hindquarter with ketone bodies.

Other differences between heart and skeletal muscle

Protein metabolism was not investigated in the present study; however, Morgan and his colleagues (see Jefferson *et al.*, 1974) have observed differences in the regulation of protein synthesis in heart and skeletal muscle. In diabetic rats, they observed ribosomal disaggregation, decreased numbers of polyribosomes and impaired protein synthesis in voluntary muscle, whereas in heart the protein synthetic apparatus was unaffected. Interestingly, they also observed that the deterioration of protein synthesis which occurs when muscle is perfused with an insulin-free medium for several hours could be prevented in heart, but not in skeletal muscle, by adding fatty acids or ketone bodies to the perfusing medium.

Although the findings of our laboratory and that of Morgan suggest significant differences in the regulation of glucose and protein metabolism in heart and skeletal muscle, it should be noted that the hind-

quarter contains a mixture of red, white and intermediate-type muscle fibres. Whether one of these fibre types resembles heart in its pattern of metabolic regulation more closely than the whole hindquarter, remains to be determined (see Cuendet *et al.*, 1975; Maizels *et al.*, 1976). In this regard, it has been shown that the enzymic apparatus of red and intermediate fibres is more like that of heart than is the enzymic apparatus of white muscle (Dawson & Romanul, 1964).

Glucose tolerance in intact animals and man

Impaired glucose tolerance has been noted in a wide variety of conditions associated with a high concentration of free fatty acids in plasma, and it has been suggested that an accelerated rate of fatty acid metabolism may play a causal role (see Hales, 1968; Ruderman *et al.*, 1969, 1976). Inhibition of glucose uptake by free fatty acids and/or ketone bodies has been noted in diaphragm (Randle *et al.*, 1966) and in mammary (Williamson, 1973) and submaxillary (Thompson & Williamson, 1975) glands as well as in heart. In addition, indirect evidence suggests that ketone bodies may inhibit the utilization of glucose by brain (Ruderman *et al.*, 1974). Of these organs only brain is a quantitatively important user of glucose. It consumes approx. 5 g of glucose/h in man; however, this occurs at a relatively constant rate and appears to be independent of changes in insulin and glucose in the physiological range. Therefore it probably contributes relatively little to glucose disposal after a carbohydrate load. For this reason, in the absence of an effect on skeletal muscle and adipose tissue (Jeanrenaud, 1961), it seems unlikely that inhibition of peripheral glucose utilization by free fatty acids or ketone bodies could cause insulin resistance or impaired glucose tolerance. Studies carried out in intact animals and man in which the plasma concentration of free fatty acids are acutely increased or decreased have been reviewed by Ruderman *et al.* (1976). Suffice it to say the direct effect of altering plasma free fatty acids on glucose metabolism in skeletal muscle was not investigated systematically in any of these studies.

In conclusion, neither free fatty acids nor ketone bodies appear to inhibit glycolysis or the uptake of glucose in the perfused rat hindquarter. On the other hand, ketone bodies, and possibly free fatty acids, inhibit the oxidation of pyruvate in skeletal muscle and ample evidence suggests that this may contribute to the decreased oxidation of glucose in skeletal muscle during starvation.

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