Biochem. J. (1975) **146**, 231–238
Printed in Great Britain

Glucose Metabolism in Perfused Skeletal Muscle

INTERACTION OF INSULIN AND EXERCISE ON GLUCOSE UPTAKE

By MICHAEL BERGER, SIGRID HAGG and NEIL B. RUDERMAN*
Elliott P. Joslin Research Laboratory and the Department of Medicine, Harvard Medical School and the Peter Bent Brigham Hospital, Boston, Mass. 02215, *U.S.A.*

(*Received* 6 *September* 1974)

1. The interaction of insulin and isometric exercise on glucose uptake by skeletal muscle was studied in the isolated perfused rat hindquarter. 2. Insulin, 10m-i.u./ml, added to the perfusate, increased glucose uptake more than 10-fold, from 0.3–0.5 to 5.2–5.4 μmol/min per 30g of muscle in hindquarters of fed and 48h-starved rats respectively. In contrast, it did not stimulate glucose uptake in hindquarters from rats in diabetic ketoacidosis. 3. In the absence of added insulin, isometric exercise, induced by sciatic-nerve stimulation, increased glucose uptake to 4 and 3.4 μmol/min per 30g of muscle in fed and starved rats respectively. It had a similar effect in rats with moderately severe diabetes, but it did not increase glucose uptake in rats with diabetic ketoacidosis or in hindquarters of fed rats that had been 'washed out' with an insulin-free perfusate. Insulin, at concentrations which did not stimulate glucose uptake in resting muscle, restored the stimulatory effect of exercise in these situations. 4. The stimulation of glucose uptake by exercise was independent of blood flow and the degree of tissue hypoxia: also it could not be reproduced by perfusing resting muscle with a medium previously used in an exercise experiment. 5. At rest glucose was not detectable in muscle cell water of fed and starved rats even when perfused with insulin. In the presence of insulin, a small accumulation of glucose, 0.25mM, was noted in the muscle of ketoacidotic diabetic rats, suggesting inhibition of glucose phosphorylation, as well as of transport. 6. During exercise, the calculated intracellular concentration of glucose in the contracting muscle increased to 1.1–1.6mM in the fed, starved and moderately diabetic groups. Insulin significantly increased the already high rates of glucose uptake by the hindquarters of these animals but it did not alter the elevated intracellular concentration of glucose. 7. In severely diabetic rats, exercise did not cause glucose to accumulate in the cell in the absence of insulin. In the presence of insulin, it increased glucose uptake to 6.1 μmol/min per 30g of muscle and intracellular glucose to 0.72mM. 8. The data indicate that the stimulatory effect of exercise on glucose uptake requires the presence of insulin. They suggest that in the absence of insulin, glucose uptake is not enhanced by exercise owing to inhibition of glucose transport into the cell.

The stimulatory effect of exercise on glucose uptake by skeletal muscle was first noted by Chauveau & Kauffman (1886), and that of insulin by Burn & Dale (1924) and by Cori *et al.* (1924). Although the independent effects of exercise and insulin on glucose transport, glycogen metabolism and glycolysis in the muscle cell have been well defined (Krahl, 1961*a*,*b*; Drummond, 1969) the interaction of these stimuli in a physiological setting is still not clarified. For instance, can exercise stimulate glucose uptake in the total absence of insulin and do exercise and insulin stimulate glucose uptake by different mechanisms? In the present study, the interaction of insulin and vigorous isometric exercise on glucose uptake was investigated in the isolated perfused rat hindquarter, a preparation composed mainly of skeletal muscle (Ruderman *et al.*, 1971). Hindquarters were exposed to various insulin concentrations or to vigorous isometric exercise induced by electrical stimulation of the sciatic nerves, or to both insulin and exercise simultaneously. Fed rats and rats with various degrees of insulin deficiency, i.e. 48h-starved, moderately diabetic and ketoacidotic, were used. A preliminary report of this work has been published (Berger *et al.*, 1973).

* To whom reprint requests should be addressed.

Materials and Methods

Animals

Female Sprague–Dawley or Wistar rats weighing 180–230g were used. Studies in which the Wistar strain was used were carried out in Oxford, U.K.; studies with the Sprague–Dawley strain were carried out in Boston, U.S.A. Animals were fed on Purina Laboratory Chow (Sprague–Dawley) or Oxoid commercial rat cubes (Wistar) *ad libitum* or were

starved for 48h before use. Severe diabetic ketoacidosis was induced by an intravenous injection of streptozotozin (125mg/kg body weight). The animals were allowed free access to food and water and were studied 72h after injection. The mean concentration ±S.E. of glucose in the tail vein of these animals at the time of surgery was 23.4±1.5mM ($n=25$) and of ketone bodies (acetoacetate plus 3-hydroxybutyrate) 6±0.2mM ($n=25$). A milder form of diabetes with less ketoacidosis was studied in rats that were killed 36h after receiving streptozotocin, and in a separate group that had received alloxan (40mg/kg body weight) intravenously after overnight starvation. Since there were no significant differences in the concentrations of glucose and ketone bodies in the blood of these groups, their results were pooled. The mean concentration of glucose±S.E.M. in the tail vein of these rats at the time of surgery was 20.9±2.8mM ($n=10$); the concentration of ketone bodies was 1.7±0.4mM ($n=10$). All procedures were carried out in rats anaesthetized with pentobarbital (3.5–5mg/100g body weight) administered intraperitoneally. Sprague–Dawley rats were used unless otherwise noted.

Materials

Bovine serum albumin (Cohn fraction V), obtained from Pentex Corp., Kankakee, Ill., U.S.A. was dissolved in Krebs–Henseleit (1932) bicarbonate solution and dialysed twice against 2 litres of the bicarbonate–saline for 24h. Streptozotocin was a gift from Dr. W. E. Dulin, Upjohn Corp., Kalamazoo, Mich., U.S.A. Glucagon-free insulin (lot no. PJ 5589) was kindly provided by Dr. W. E. Shaw, Eli Lilly Corp., Indianapolis, Ind., U.S.A. Alloxan was obtained from Sigma, St. Louis, Mo., U.S.A., and D-[1-^{3}H]sorbitol from New England Nuclear Corp., Boston, Mass., U.S.A. All enzymes and cofactors used in metabolite assays were purchased from Boehringer Corp., New York, N.Y., U.S.A., or from Sigma.

Perfusion procedure

The operative preparation of the hindquarter and the perfusion apparatus has been described by Ruderman *et al.* (1971). During the operative preparation, the skin of the rat was partially removed from both legs to permit biopsy of the thigh and calf muscle *in situ*, and Dastre's electrodes were attached to the sciatic nerves (Ruderman *et al.*, 1971). The legs were fixed to the perfusion platform at the ankles with adhesive tape. Flow rate through the tissue was measured with a Gilmont flow meter (glass float; Arthur H. Thomas, Philadelphia, Pa., U.S.A.). Unless otherwise stated, the preliminary 'washout period' was eliminated and the hindquarter was cyclically perfused with 150ml of standard medium as soon as it was placed in the apparatus. The perfusate was composed of Krebs–Henseleit solution containing 4% bovine serum albumin, aged washed human erythrocytes (7–8g of haemoglobin/100ml), 6.5–7mM-glucose, 0.15mM-pyruvate and 1.5–2.0mM-lactate. In some experiments sorbitol, 30mg/100ml, and [1-^{3}H]sorbitol, 10μCi, were added to determine the extracellular space.

After 10min of perfusion samples were collected for the determination of zero values. Glucose uptake and lactate release were determined during the subsequent 15min with the hindquarter at rest and then for a second 15min during which the hindquarter was electrically stimulated. Isometric contractions were induced by square-wave electrical pulses from a Grass SK2 stimulator. The pulses were applied for a duration of 1ms with a frequency of 5/s. A voltage of 0.1–0.5V was used at the beginning of the exercise period; however, considerable increases in the voltage (up to 5V) were sometimes necessary to maintain the intensity of the contractions at the end of the 15min period. The intensity of the contractions was assessed during the experiment by palpation of the exercising muscles. Approximately one-third to one-half of the muscle tissue of the hindquarter contracted intensely when the nerves were stimulated (Houghton, 1971). Contractions of lesser intensity occurred in the remaining leg musculature, presumably owing to direct stimulation of the muscle by the electrodes. Exercise caused minor changes in flow rate, but by slightly adjusting the 'arterial' pressure of the system, flow rate was kept constant at 11ml/min throughout. The mean pressure in the tubing leading to the aortic cannula was 8.0–9.3kPa (60–70mmHg).

Sampling of perfusate and tissue

Samples of perfusate were taken at 0, 15 and 30min. At the end of each perfusion (i.e. at 15 or 30min), a biopsy containing gastrocnemius and posterior inferior thigh muscles was obtained with specially constructed aluminum clamps, cooled in liquid N_2 (Ruderman *et al.*, 1971). When sorbitol had been added to determine the extracellular space, a sample of perfusate was centrifuged to remove erythrocytes, deproteinized and counted for radioactivity as described below.

Analytical methods

Perfusate and tissue specimens (after being ground in a cooled percussion mortar) were deproteinized in ice-cold 10% (w/v) $HClO_4$. The extracts were neutralized with KOH and the precipitated $KClO_4$ was removed by centrifugation. The following metabolites were measured spectrophotometrically

with standard enzymic assays: glucose (Slein, 1963), lactate (Hohorst, 1963), ATP (Lamprecht & Trautschold, 1963) and creatine phosphate (Lamprecht & Stein, 1963). Radioactivity due to [^{3}H]sorbitol was counted in deproteinized and neutralized samples of tissue and perfusate plasma on a Nuclear–Chicago Isostat-300 liquid-scintillation counter. The O_2 content of whole perfusate was determined manometrically in arterial and venous samples by the method of Van Slyke & Neill (1924). O_2 consumption was determined from the arteriovenous difference in O_2 content and the flow rate at the mid-point of the experimental period.

Calculations

Glucose uptake, lactate release and O_2 consumption by the hindquarter are expressed as μmol/min per 30g of muscle (Ruderman *et al.*, 1971). The extracellular space (E) was measured with [1-^{3}H]-sorbitol as marker as indicated in eqn. 1:

$$\text{E(ml/g)} = \frac{\text{d.p.m./g of tissue}}{\text{d.p.m./ml of perfusate plasma water}} \quad (1)$$

The concentration of glucose in muscle cell water was calculated as shown in eqn. 2:

$$Ci = \frac{Cm-(Cp\times E)}{1-\text{E}} \times \frac{1-\text{E}}{0.8-\text{E}} = \frac{Cm-Cp\times \text{E}}{0.8-\text{E}} \quad (2)$$

(cf. Ruderman & Goodman, 1973) where Ci = μmol of glucose/ml in cell water; Cm = μmol of glucose/g wet weight of muscle; and Cp = μmol of glucose/ml of perfusate plasma water. It was assumed that muscle contains 80% water and that the concentration of glucose in interstitial water is equal to its concentration in plasma water (see below). The concentration of glucose in venous plasma water (Cp) was calculated from the concentration of glucose in whole perfusate in the collecting flask, i.e. in arterial perfusate, as indicated in eqn. 3. Here C_b = glucose concentration in whole perfusate in mM; glucose uptake was calculated from C_b during a 15min experimental period and the perfusate volume. The term 1.11 is the ratio between the glucose concentration in perfusate plasma water and whole perfusate, determined in eight experiments (1.11±0.02 S.E.M.).

$$Cp = C_b - \frac{\text{glucose uptake in } \mu\text{mol/min}}{\text{flow rate in ml/min}} \times 1.11 \quad (3)$$

Results

Glucose uptake by resting muscle

Effect of insulin and glucose concentration. In order to select the optimal conditions for studying glucose metabolism, we assessed glucose uptake by the hindquarter in the presence of various concentrations of insulin and glucose. Insulin, at concentrations as low as 0.2m-i.u./ml, significantly increased the uptake of glucose in hindquarters of fed rats; however, the effect of the hormone at this concentration was delayed and glucose uptake was increased only during the second 15min of perfusion (Table 1). A maximum effect of the hormone (4.9±1.1 μmol/min per 30g of muscle), evident during both the initial and second

Table 1. *Effect of perfusate insulin concentration on glucose uptake by the isolated rat hindquarter*

Results are means±S.E.M. with numbers of experiments in parentheses. Hindquarters of fed rats were perfused with 150ml of standard medium. The first 50ml of perfusate which passed through the tissue was discarded and the perfusate was then recycled. Insulin was added at the beginning of the recycling period: glucose uptake was measured during the subsequent 30min.

Insulin added to perfusate (m-i.u./ml)		Glucose uptake (μmol/min per 30g of muscle) 0–15min	15–30min
0	(4)	0.4±0.8	0.1±0.4
0.2	(12)	0.5±0.4	3.0±0.6†§
5.0	(3)	2.1±0.7	5.3±0.3‡‖
10	(6)	4.9±1.1*	3.6±0.5‡

* Value significantly different from that of the hindquarter perfused without added insulin, $P<0.05$.
† As for *, $P<0.01$.
‡ As for *, $P<0.001$.
§ Value significantly different from that obtained during the initial 15min of perfusion, $P<0.05$.
‖ As for §, $P<0.01$.

Table 2. *Effect of perfusate glucose concentration on glucose uptake by the isolated rat hindquarter*

Results are means±S.E.M. with numbers of observations in parentheses. Hindquarters of 48h-starved Wistar rats were perfused with 200ml of standard perfusate containing 12.5m-i.u. of insulin/ml. The first 75ml of perfusate was discarded and the medium was then recycled. After 5min of recycled perfusion, glucose uptake was determined during two consecutive 15min intervals. Data from both periods are included in the results.

Initial perfusate glucose concn. (mM)	Glucose uptake (μmol/min per 30g of muscle)
3–4	5.3±0.7 (7)
4–5	6.6±0.6 (10)
5–6	7.5±0.9 (3)
6–8	10.0±1.4 (3)
8–10	10.7±0.5 (3)
16–20	13.4±2.3 (4)

15min of perfusion, was obtained only when 10m-i.u. of insulin/ml or more was added to the medium.

The magnitude of glucose uptake was also affected by perfusate glucose concentration. When hindquarters were perfused with a high concentration of insulin, the utilization of glucose increased markedly as the perfusate glucose concentration was increased from 3 to 20mM (Table 2). Although not evident from the presented data, glucose uptake was comparable during the first and second 15min experimental periods, suggesting that equilibration between the vascular and interstitial spaces of muscle did not materially alter the results. On the basis of these experiments, the concentration of glucose in the initial perfusate was set at 6.5mM and the concentration of insulin (when it was added) at 10m-i.u./ml in most subsequent experiments.

Effect of starvation and diabetes. In all groups the uptake of glucose by resting muscle was negligible in the absence of added insulin (Table 3). Insulin increased the uptake of glucose in resting muscle to 5.2 ± 0.4 and $5.4\pm0.7\mu$mol/min per 30g of muscle in fed and starved rats respectively. In contrast, it did not significantly increase the uptake of glucose in rats with diabetic ketoacidosis.

Glucose uptake by exercising muscle

Comparability of exercise in the different groups. During a perfusion, the 'intensity' of isometric exercise was assessed by palpation from the tension of the hindlimb muscle during a contraction. That the intensity of exercise was comparable in the different groups is suggested by the finding that increases in tissue lactate and decreases in ATP and creatine phosphate were similar in all groups (Table 4: data for starved and moderately diabetic groups not shown). In addition, O_2 consumption, which was increased from 7.6 ± 0.5 ($n=4$) to 23.4 ± 2.4 ($n=5$) μmol/min per 30g of muscle in fed rats, was increased to a similar value in starved ($20.7\pm4.6\mu$mol/min per 30g of muscle, $n=3$) and severely diabetic ($24.3\pm3.4\mu$mol per 30g of muscle, $n=4$) rats. Lactate release during exercise was also comparable, ranging between 9.6 and 11.5μmol/min per 30g of muscle, in the fed and starved groups (versus 1–2μmol/min per 30g of muscle at rest), with insulin having no significant effect. Lactate release was slightly higher in the severely diabetic rats: 16.5 ± 1.7 ($n=9$) and 12.8 ± 1.5 ($n=9$) μmol/min per 30g of muscle in animals perfused with and without insulin respectively.

Glucose uptake. In fed and starved rats, exercise caused an 8–12-fold increase in glucose uptake when insulin was not added to the perfusate (Table 3). This is in accordance with earlier work carried out with exercising hindlimb and hindquarter preparations (McGuigan, 1908; Huycke & Kruhoffer, 1955; Szabo *et al.*, 1969; Ruderman *et al.*, 1971). In the presence of added insulin, exercise caused a further increase in the uptake of glucose from 5.2 ± 0.4 to $8.4\pm0.6\mu$mol/min per 30g of muscle in fed rats and from 5.4 ± 0.7 to $8.8\pm0.6\mu$mol/min per 30g of muscle in starved rats. The latter values are significantly higher than those obtained with insulin alone,

Table 3. *Effects of exercise and insulin on glucose uptake by the perfused hindquarter of fed, starved and diabetic rats*

Hindquarters in most experiments were perfused for 15min at rest, and were then exercised isometrically for 15min by sciatic-nerve stimulation (5/s). In those studies in which the tissue was washed out, glucose uptake was determined during the initial 15min.

State of rat	Insulin (10m-i.u./ml)	Glucose uptake (μmol/min per 30g of muscle) Resting muscle	Exercising muscle
Fed	−	0.5 ± 0.2 (34)	4.0 ± 0.3 (17)‖
	+	5.2 ± 0.4 (18)†	8.4 ± 0.6 (10)†§
	−¶	—	4.1 ± 0.5 (6)
Starved for 48h	−	0.3 ± 0.3 (8)	3.4 ± 0.6 (6)§
	+	5.4 ± 0.7 (8)†	8.8 ± 0.6 (6)†§
Moderate diabetes	−	1.2 ± 0.6 (10)	3.5 ± 0.6 (10)‡
Severe diabetes	−	-0.5 ± 0.3 (11)	-0.7 ± 0.5 (10)
	+	1.5 ± 1.1 (9)	6.1 ± 1.1 (9)†§
	0.2m-i.u./ml		
Fed(prior washout)	−	—	0.5 ± 0.6 (5)
	+	0.5 ± 0.4 (12)	3.0 ± 0.6 (4)*§

* Value significantly different from that of hindquarter perfused without insulin, $P<0.01$.
† As for *, $P<0.001$.
‡ Value different from that in resting muscle $P<0.05$.
§ As for ‡, $P<0.01$.
‖ As for ‡, $P<0.001$.
¶ Flow rate maintained at 25ml/min.

Table 4. *Effect of exercise on tissue concentrations of lactate, ATP and creatine phosphate*

Results are means±S.E.M. with numbers of experiments in parentheses. Hindquarters were perfused with 150ml of standard medium and tissue was biopsied at the end of the perfusion period, as described in the Materials and Methods section. R, resting muscle; E, muscle performing isometric exercise at a rate of 5/s.

		Tissue concentration (μmol/g wet weight)					
		Lactate		ATP		Creatine phosphate	
State of rat	Insulin (10m-i.u./ml)	R	E	R	E	R	E
Fed							
(18)	−	1.7±0.2	14.0±1.0	4.9±0.1	3.8±0.2	14.7±0.5	5.9±0.7
(6)	+	1.9±0.2	11.8±1.2	4.8±0.2	3.7±0.2	18.6±1.1	5.3±0.8
(6)	−*	—	6.5±1.1	—	4.2±0.2	—	7.0±0.9
Severe diabetes							
(11)	−	1.6±0.3	10.6±1.2	5.4±0.4	3.8±0.2	14.2±0.7	6.2±0.8
(10)	+	2.5±0.4	10.7±1.0	4.8±0.4	3.7±0.2	14.5±0.6	5.8±0.7

* Flow rate maintained at 25ml/min instead of the usual rate of 11ml/min.

indicating that the effect of exercise is additive. It is also noteworthy that lactate production was not enhanced by insulin (see above), as this suggests that the additional glucose removed by the hindquarter was converted into glycogen and/or that glycogen breakdown was depressed.

Requirement for insulin. Exercise enhanced the uptake of glucose by hindquarters of moderately diabetic rats (Table 2). The latter probably were not completely devoid of insulin since the total ketone-body concentration in their blood averaged only 1.7mM. In contrast, exercise did not stimulate glucose uptake in rats with diabetic ketoacidosis (total ketone-body concentration about 6mM), which are presumably totally insulin-deficient. When insulin, which was ineffective in resting muscle, was added to the medium perfusing these severely diabetic animals the ability of exercise to stimulate glucose uptake was restored.

Effect of 'washing out' the hindquarter. When the first 70ml of perfusate passing through the hindquarter was discarded before the medium was recycled, the stimulatory effect of exercise on glucose uptake was lost (Table 3). Presumably a substance present in the tissue and required for exercise to enhance glucose uptake was washed out. The addition to the perfusate of 0.2m-i.u. of insulin/ml did not enhance glucose uptake in resting muscle, but it restored the stimulatory effect of exercise.

Hypoxia and glucose uptake. The perfused rat heart, the isolated rat diaphragm (Randle & Smith, 1958; Morgan *et al.*, 1961*a*) and the frog sartorius muscle (Ozand *et al.*, 1962) increase their rates of glucose uptake during anoxia. To investigate whether hypoxia may have contributed to the increased glucose uptake of the hindquarter during exercise, the flow of perfusate to the tissue was increased 2.5-fold by increasing the arterial pressure from 9.3 to 17.3kPa (70–130 mmHg). This resulted in an increased uptake of O_2 by the hindquarter from 23.4±2.4 ($n=5$) to 32.4±1.5μmol/min per 30g of muscle ($n=4$) in fed rats and it markedly increased the pO_2 of the venous blood leaving the muscle. In addition, it resulted in a lesser increase of tissue lactate and a slightly diminished fall of tissue ATP and creatine phosphate (Table 4). The stimulatory effect of exercise on glucose uptake was not affected, however.

Muscular activity factor. It has been suggested that exercising muscle releases a substance into the blood ('muscular activity factor') which can increase glucose uptake in resting muscle (Goldstein *et al.*, 1953; Goldstein, 1961). To determine whether such a substance is released into the perfusate, hindquarters maintained in the resting state were perfused with media used in a prior exercise study. Glucose uptake [0.5±0.3μmol/min per 30g of muscle ($n=6$)] was not increased; however, this does not exclude the possibility that 'muscle activity factor' was released locally, as it could have escaped detection because of dilution by the approx. 150ml of perfusate.

Glucose transport and accumulation. The magnitude of glucose uptake by muscle is determined by the rate at which glucose is transported into the cell and subsequently phosphorylated (Morgan *et al.*, 1961*a*). To study the interaction of insulin and exercise on these processes, we determined their effect on the concentration of free glucose within the muscle of the hindquarter. The extracellular space, determined from the distribution of [1-^{3}H]sorbitol between perfusate plasma water and tissue, was 200±20μl/g wet wt. (mean±S.E.M., $n=5$) in resting muscle and 200±30μl/g wet wt. (mean ±S.E.M., $n=5$) during exercise. In the absence of added insulin, glucose was confined to the extracellular space in resting muscle (Table 5), suggesting that transport was rate-limiting. Insulin caused a small but significant accumulation of

Table 5. *Effect of insulin and exercise on the concentrations of glucose and glucose 6-phosphate in muscle*

Results are means±S.E.M. with numbers of experiments in parentheses. See the Materials and Methods section for details of calculations.

State of rat	Insulin (10m-i.u./ml)	Calculated glucose concentration in muscle cell water (μmol/ml)		Glucose 6-phosphate (μmol/g wet wt.)	
		Rest	Exercise	Rest	Exercise
Fed	−	n.d.* (19)	1.25±0.15 (19)	0.32±0.04 (7)	1.01±0.19 (7)
	+	n.d. (8)	1.04±0.24 (9)	0.57±0.12 (6)	0.88±0.16 (7)
Starved for 48h	−	n.d. (6)	1.11±0.17 (8)	0.20±0.01 (6)	0.60±0.12 (7)
	+	n.d. (6)	0.67±0.24 (11)	0.29±0.03 (6)	0.64±0.08 (9)
Moderate diabetes	−	n.d. (4)	1.59±0.22 (10)	—	—
Severe diabetes	−	n.d. (6)	n.d. (11)	0.27±0.04 (6)	0.39±0.07 (9)
	+	0.26±0.06 (6)	0.72±0.22 (8)	0.30±0.07 (6)	0.49±0.08 (8)

* Glucose distributed in a volume less than the extracellular space of muscle (i.e. <20%).

glucose in the severely diabetic group, suggesting inhibition of glucose phosphorylation as well as transport in these animals. It did not cause glucose to accumulate in the muscle of fed or starved rats; however, the estimation of muscle cell glucose is problematical in these animals, since the concentration of glucose in the interstitial fluid was not determined. Glucose in cell water was calculated on the assumption that the concentrations of glucose in perfusate water and in the interstitial fluid bathing the muscle were very similar. If the glucose concentration in the interstitial fluid were one-half that in the perfusate water, the concentration of glucose in cell water would be underestimated by approx. 0.7μmol/ml. Such an occurrence could conceivably take place when glucose transport is stimulated by insulin, if the transfer of glucose from the vascular space to the muscle cell membrane is not very rapid (Morgan *et al.*, 1961*a*). Whether this actually occurs is not known.

In fed, starved and moderately diabetic rats, exercise caused glucose to accumulate in the muscle cell, as first noted by Cori *et al.* (1933). The observed intracellular concentrations of glucose (1–1.6mM) in the intensely contracting muscle were more than 10-fold greater than the K_m of muscle hexokinase for glucose (9×10^{-5}M) (Ozand *et al.*, 1962). Cell glucose was not significantly altered when these hindquarters were perfused with insulin. Under no conditions did glucose accumulate in the portions of the hindquarter that were not intensely contracting (see the Materials and Methods section).

Exercise did not increase cell glucose in the muscle of severely diabetic rats, suggesting that its failure to enhance uptake in these animals was due to a block in glucose transport. When insulin was added to the perfusate, exercise both stimulated glucose uptake (Table 3) and caused it to accumulate within the muscle cell (Table 5).

Glucose 6-phosphate. In accordance with earlier reports (see Sacktor *et al.*, 1965; Kirsten *et al.*, 1969), vigorous exercise caused a severalfold increase in the concentration of glucose 6-phosphate in the muscle of fed and starved rats. It had a lesser effect in severely diabetic rats. Insulin slightly increased the concentration of glucose 6-phosphate in the hindquarters of fed and starved rats during rest, but it had no effect in diabetic rats. It also did not alter the increase in glucose 6-phosphate caused by exercise.

Discussion

The data suggest that the stimulation of glucose uptake by exercise requires the presence of insulin. Whereas muscle contraction enhanced glucose uptake in hindquarters of fed, starved and moderately diabetic rats, it had no effect in rats in diabetic ketoacidosis or in fed rats when the hindquarter was 'washed out' with an insulin-free perfusate before stimulation. Further, in both situations in which exercise was ineffective, the addition of insulin to the perfusate restored its stimulatory effect on glucose uptake.

The concentration of insulin required for exercise to stimulate glucose uptake is undoubtedly very low. In experiments in which tissue was cyclically perfused with an insulin-free medium, the sole source of insulin would have been the blood and interstitial fluid present in the tissue when it was placed in the perfusion apparatus, plus whatever insulin was bound to the tissue. We have found approx. 5 and 30m-i.u. of immunoassayable insulin/ml of the sera of 48h-starved and fed rats respectively (M. Berger, S. Hagg & N. B. Ruderman, unpublished work); therefore the concentrations in the interstitial space (volume 5–6ml) after equilibration with 150ml of a perfusate devoid of insulin, would have ranged between 0.2 and 1.2μ-i.u./ml. This is far less than the minimal effective concentration of insulin which stimulates glucose

uptake in the perfused hindquarter at rest (100–200μ-i.u./ml: Strohfeldt *et al.*, 1974; the present paper).

Small amounts of insulin (<10μ-i.u./ml) are also required for anoxia to stimulate glucose uptake in skeletal muscle (Gould & Chaudry, 1970). It has been proposed that insulin 'primes' the muscle cell membrane so that it can respond to anoxia; however, the nature of the 'priming' effect is not clear. Other explanations could also account for the requirement for insulin. For instance, exercise and anoxia might increase the affinity of receptors on the muscle cell membrane for insulin, so that even at very low concentrations, a sufficient amount of insulin could be bound to stimulate glucose uptake. Likewise, insulin could in some way be necessary for the generation of 'muscle activity factor'. Although the physiological role and indeed the very existence of 'muscle activity factor' have been challenged by investigators unable to find it in the blood of man (Sanders *et al.*, 1964) and experimental animals (Helmreich & Cori, 1957; Dulin & Clark, 1961) during exercise, Havivi & Wertheimer (1964) and others (R-Candela & R-Candela, 1962; Fredrickson *et al.*, 1969) have readily demonstrated it in the medium incubating diaphragm and skeletal muscle stimulated *in vitro*. In addition, Coutourier *et al.* (1971) have noted a marked increase in non-suppressible insulin-like activity in the lymph draining a dog hindlimb during muscle contraction, but were unable to detect it in blood. Collectively, these findings suggest that 'muscle activity factor' is released locally, and that it could escape detection if sufficiently diluted or inactivated before reaching the systemic circulation. This could explain the inability of Szabo *et al.* (1972) and our group (this study) to demonstrate muscle 'activity factor' in the medium perfusing a rat hindquarter. Whether the generation of 'muscle activity factor' is required for exercise to stimulate glucose uptake is not known; therefore it would be of great interest to determine if it can be produced by the muscle of a rat in diabetic ketoacidosis, and if not, whether insulin would allow it to appear.

The requirement for insulin to demonstrate the stimulatory effect of exercise on glucose uptake is in accordance with earlier data obtained in man and experimental animals. Before the introduction of insulin therapy, it was observed that exercise intensified hyperglycaemia in patients with diabetic ketosis, whereas it lowered the blood sugar in patients with more moderate diabetes (Allen *et al.*, 1919). Later, Marble & Smith (1936) noted that exercise caused an increase in blood glucose in patients with juvenile diabetes when insulin was withheld, whereas in the patient receiving adequate insulin it lowered the blood glucose. It has also been demonstrated that exercise does not stimulate glucose uptake in the forearm of patients with juvenile diabetes 16h after insulin withdrawal (Whichelow *et al.*, 1968). Similar results have been obtained in studies carried out in dogs (Seo, 1908; Yater *et al.*, 1933). The sporadic reports (Seo, 1908; Ingle *et al.*, 1951; Sanders *et al.*, 1964; Moxness *et al.*, 1964; Lyngse *et al.*, 1973) that suggest that exercise lowers blood glucose in diabetic animals and man can probably be explained by the fact that experimental subjects were not totally deficient in insulin.

In addition to allowing exercise to stimulate glucose uptake, insulin at high concentrations independently stimulated glucose utilization by the exercising hindquarter. This occurred when the intracellular concentration of glucose in the intensely contracting muscle was more than 10-fold greater than the K_m of muscle hexokinase for glucose. Thus, if insulin stimulated glucose uptake into this tissue (which accounts for one-third of the mass of the hindquarter) it did so by virtue of an effect on intracellular glucose disposition. Increments in glucose uptake when the concentration of glucose in the muscle cell is far greater than the K_m for glucose of hexokinase have been previously noted in rat heart perfused with insulin (Morgan *et al.*, 1961*b*) and in anoxic frog muscle incubated with insulin (Ozand *et al.*, 1962). Experiments in which 100% of the muscle is made to contract, in either the hindquarter or another muscle preparation, should resolve the question whether insulin has a similar effect on exercising skeletal muscle.

The results support the hypothesis that insulin has a dual effect on glucose uptake by skeletal muscle (Gould & Chaudry, 1970). Large amounts of insulin have a modulatory role, in that the ambient concentration of insulin specifically determines the rate of glucose uptake. In contrast, small amounts of insulin, which do not stimulate glucose uptake in resting muscle, are required for the stimulatory effect of exercise and anoxia. Here the role of insulin may be 'permissive' in that insulin appears to be maintaining or priming the sugar-transport system rather than modulating it, although as noted above, the latter possibility has not been ruled out.

We thank Professor G. F. Cahill, Jr., for reviewing the manuscript. The technical assistance of Miss Helena Pavlincova, Miss Heather Jones, Mrs. Zenta Skulte and Mrs. Ludmilla Klavins is gratefully acknowledged. These studies were carried out in part at the Metabolic Research Laboratory, Nuffield Department of Medicine, Oxford, U.K. They were supported in part by grants to Professor H. A. Krebs from the U.S. Public Health Service (AM 11748-03), the Medical Research Council and the British Diabetes Association and by U.S. Public Health Service Grants AM-05077, AM-15191 and AM-15272. N. B. R. was the recipient of a Research and Development Award from the American Diabetes Association and of a Career Research and Development Award from the National Institutes of Health (KO4-AM 15729-02). M. B. was the recipient of grants from the German Research Association and the German Diabetes Association.

References

Allen, F. M., Stillman, E. & Fitz, R. (1919) *Total Dietary Regulation in the Treatment of Diabetes* (chapter 5), monograph no. 11, pp. 468–499, The Rockefeller Institute for Medical Research, New York

Berger, M., Hagg, S. A. & Ruderman, N. B. (1973) *Diabetes* **22** (*Suppl.* **1**), 292

Burn, J. H. & Dale, H. H. (1924) *J. Physiol.* (*London*) **59**, 164–192

Chauveau, M. A. & Kaufmann, M. (1886) *C. R. Acad. Sci. Ser. D* **104**, 1126–1132

Cori, C. F., Cori, G. T. & Goltz, H. L. (1924) *J. Pharmacol. Exp. Ther.* **22**, 355–373

Cori, G. T., Closs, J. O. & Cori, C. F. (1933) *J. Biol. Chem.* **103**, 13–24

Couturier, E., Rasio, E. & Conard, V. (1971) *Horm. Metab. Res.* **3**, 382–286

Drummond, G. I. (1969) *Forts. Zool.* **18**, 359–429

Dulin, W. E. & Clark, J. J. (1961) *Diabetes* **10**, 289–297

Fredickson, R. C. A., Bihler, I. & Fresel, P. E. (1969) *Can. J. Physiol. Pharmacol.* **47**, 216–218

Goldstein, M. S. (1961) *Amer. J. Physiol.* **200**, 67–70

Goldstein, M. S., Mullick, V., Huddlestun, B. & Levine, R. (1953) *Amer. J. Physiol.* **173**, 212–216

Gould, M. K. & Chaudry, J. H. (1970) *Biochim. Biophys. Acta* **215**, 258–263

Havivi, E. & Wertheimer, H. E. (1964) *J. Physiol.* (*London*) **172**, 342–352

Helmreich, E. & Cori, C. F. (1957) *J. Biol. Chem.* **224**, 663–679

Hohorst, H. J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 266–270, Academic Press, New York and London

Houghton, C. R. S. (1971) D.Phil. Thesis, University of Oxford

Huycke, E. J. & Kruhoffer, P. (1955) *Acta Physiol. Scand.* **34**, 232–249

Ingle, D. J., Nezamis, J. E. & Morley, E. H. (1951) *Amer. J. Physiol.* **165**, 469–472

Kirsten, R., Kirsten, E. & Wolff, J. (1969) *Pflügers Arch.* **307**, 154–166

Krahl, M. (1961*a*) *Action of Insulin on Cells*, pp. 15–40, Academic Press, New York and London

Krahl, M. (1961*b*) *Action of Insulin on Cells*, pp. 81–108, Academic Press, New York and London

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66

Lamprecht, W. & Stein, P. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H.U., ed.), pp. 610–616, Academic Press, New York and London

Lamprecht, W. & Trautschold, J. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 543–551, Academic Press, New York and London

Lyngsøe, J., Clausen, J., Dambard, S., Nielsen, S. L., Rehfeld, J., Schaffalitzky de Muckadell, O., Sestoft, L. & Trap-Jensen, J. (1973) *Excerpta Med. Found. Int. Congr. Ser. no.* 280, p. 97, Amsterdam

Marble, A. & Smith, A. B. (1936) *Arch. Intern. Med.* **58**, 577–588

McGuigan, H. (1908) *Amer. J. Physiol.* **21**, 334–350

Morgan, H. E., Henderson, M. J., Regen, D. M. & Park, C. R. (1961*a*) *J. Biol. Chem.* **236**, 253–261

Morgan, H. E., Cadenas, E., Regen, D. M. & Park, C. R. (1961*b*) *J. Biol. Chem.* **236**, 262–268

Moxness, K. E., Molnar, G. D. & McGuckin, W. F. (1964) *Diabetes* **13**, 38–43

Ozand, P., Narahara, H. T. & Cori, C. F. (1962) *J. Biol. Chem.* **237**, 3037–3043

R-Candela, R. & R-Candela, Y. (1962) *Proc. Soc. Exp. Biol. Med.* **110**, 803–804

Randle, P. J. & Smith, G. H. (1958) *Biochem. J.* **70**, 501–508

Ruderman, N. B. & Goodman, M. N. (1973) *Amer. J. Physiol.* **224**, 1391–1397

Ruderman, N. B., Houghton, C. R. S. & Hems, R. (1971) *Biochem. J.* **124**, 639–651

Sacktor, B., Wormser-Shavit, E. & White, J. I. (1965) *J. Biol. Chem.* **240**, 2678–2681

Sanders, C. A., Levinson, G. E., Abelmann, W. H. & Freinkel, N. (1964) *N. Engl. J. Med.* **271**, 220–225

Seo, Y. (1908) *Arch. Exp. Pathol.* **59**, 341–363

Slein, M. W. (1963) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), pp. 118–123, Academic Press, New York and London

Strohfeldt, P., Kettel, H. & Weinges, K. F. (1974) *Horm. Metab. Res.* **6**, 167–168

Szabo, A. J., Mahler, R. J. & Szabo, O. (1969) *Horm. Metab. Res.* **1**, 156–161

Szabo, O., Szabo, A. J. & Mahler, R. J. (1972) *Horm. Metab. Res.* **4**, 139–143

Van Slyke, D. D. & Neill, J. M. (1924) *J. Biol. Chem.* **61**, 523–573

Whichelow, M. J., Butterfield, W. J. H., Abrams, M. E., Sterky, G. & Garratt, C. J. (1968) *Metab. Clin. Exp.* **17**, 84–96

Yater, W. M., Markowitz, J. & Cahoon, R. F. (1933) *Arch. Intern. Med.* **51**, 800–813