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Sensitivity to insulin of glycolysis and glycogen synthesis of isolated soleus-muscle strips from sedentary, exercised and exercise-trained rats

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1. The half-maximal stimulation of the rates of glycolysis and glycogen synthesis in soleus-muscle strips from sedentary animals occurred at a concentration of insulin of about 100μunits/ml. 2. In soleus-muscle strips from exercise-trained rats (5 weeks of treadmill training), half-maximal stimulation of the rate of glycolysis occurred at about 10μunits of insulin/ml, whereas that for glycogen synthesis occurred between 10 and 100μunits of insulin/ml. The sensitivity of glycolysis to insulin after exercise training is similar to that of adipose tissue from sedentary animals. This finding suggests that, in sedentary animals, the effects of normal changes in insulin concentration may affect muscle primarily indirectly via the anti-lipolytic effect on adipose tissue, whereas after training insulin may effect the rate of glycolysis in muscle directly. 3. A single period of exercise did not change the sensitivity of glycolysis in soleus muscle to insulin, nor probably that of glycogen synthesis. 4. It is suggested that the improvement in insulin sensitivity of glycolysis in muscle caused by exercise-training could account, in part, for the well-established improvement in glucose tolerance and insulin sensitivity observed in man and rats after exercise-training.

Adipose tissue and muscle are two of the major tissues of the body that respond acutely to insulin by increasing their rates of glucose uptake and utilization. It has been shown with isolated adipocytes that not only glucose uptake but also lipolysis are similarly sensitive to insulin, with a half-maximal effect at approx. 10 μunits/ml) (Green & Newsholme, 1979). However, there have been few systematic studies on the sensitivity of glucose utilization of muscle to insulin. Studies with the perfused isolated hindlimb of the rat and studies *in vivo* on the forearm muscle of man indicate a half-maximal effect of insulin at much higher concentrations (150–400 μunits/ml) than those observed with adipocytes (Richter *et al.*, 1982; Zierler & Rabinowitz, 1964). A difference in sensitivity between muscle and adipose tissue may be of physiological importance in understanding how insulin controls the blood glucose concentration since, if adipose tissue is more sensitive, low concentrations of the hormone would influence glucose metabolism in muscle, mainly indirectly via its effect on fatty acid mobilization from adipose tissue (Newsholme, 1976, 1977; Green & Newsholme, 1979).

Exercise-training in man and rats has been shown to improve glucose tolerance and to lower the insulin concentrations in response to an oral or intravenous glucose load (Bjorntorp *et al.*, 1972; Berger *et al.*, 1979). This indicates that insulin sensitivity has been increased in the intact animal, but it is unclear which tissue(s) had been affected by the training. In order to investigate the sensitivity of skeletal muscle to insulin, the effect of insulin concentration on the rates of glycolysis and glycogen synthesis in thin strips of soleus muscle of the rat incubated *in vitro* have been measured in sedentary and exercise-trained animals and in sedentary animals immediately after a single period of exercise. The results are reported and discussed in this paper.

Materials and methods

Animals, chemicals and enzymes were obtained from the sources given previously (Green & Newsholme, 1979), except for [U-^{14}C]glucose, which was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Exercise and training

A group of sedentary rats was subjected to a single period of exercise, and these are termed

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exercised rats: they were run on a treadmill for 90 min at a speed of 28 m/min. The rats were killed within 1 h of exercise, and muscles were removed immediately for incubation. Exercise-trained rats were run daily for 6 days a week on a treadmill at 28 m/min for a period of 4 weeks: at first, exercise lasted for 15 min, but this was increased by 5 min each day until the duration was 60 min. This period was maintained each day for the remainder of the training programme. These rats were not exercised on the day of the experiment. Sedentary control rats were not subjected to exercise.

Incubation of strips of soleus muscle

Rats were killed by cervical dislocation, and the soleus muscle from each leg was carefully exposed. The soleus muscle was divided into two strips as described by Crettaz *et al.* (1980). In some cases one rat provides four strips, but sometimes only two strips were obtained. The strips, which weigh between 25 and 35 mg, were then attached to stainless-steel clips and transferred directly to 4 ml of Krebs–Ringer bicarbonate buffer (Krebs & Henseleit, 1932), pH 7.4, containing 1% (w/v) albumin [which had been defatted as described by Chen (1967) and dialysed overnight against the buffer] and 5 mM-glucose in a 25 ml silicone-treated Erlenmeyer flask at 37°C. The buffer had been previously gassed with O_2/CO_2 (19:1) for 30 min. The strips were preincubated for 30 min, transferred to another flask containing the same medium except for the presence of [^{14}C]glucose (0.25 μCi/ml) and insulin at concentrations between 0 and 10 munits/ml (see Figures for details) and incubated for a further period of 60 min. The flasks were gassed continuously during the period of preincubation and also for the first 10 min of the second incubation. At the end of the incubation the muscle strips were removed and immediately freeze-clamped; to a volume of incubation medium sufficient 40% (w/v) $HClO_4$ was added to give a final concentration of 40% (w/v), and the protein was removed by centrifugation (1200 *g* for 5 min): the supernatant was neutralized with KOH and precipitated $KClO_4$ removed by centrifugation (1000 *g* for 10 min). Lactate was assayed enzymically in the supernatant by the method of Gutman & Wahlefeld (1974) and in some experiments radiochemically by separation of lactate on an ion-exchange column (Hammerstedt, 1980). When the concentration was measured radiochemically and enzymically in the same experiment, the two methods gave similar results (within 10%). The radioactivity incorporated into glycogen was measured as described by Cuendet *et al.* (1976); the freeze-clamped muscle was extracted in 0.5 ml of 1 M-NaOH for 1 h. A volume (1.5 ml) of 75% (v/v) ethanol was added to the extract and left overnight. The precipitated glycogen was redissolved in 0.5 ml of water and re-precipitated. The precipitate was dissolved in water and a sample dissolved in 10 ml of scintillant [which contained 2.0 g of 2,5-diphenyloxazole and 0.05 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 500 ml of toluene plus 250 ml of Triton X-100 (Patterson & Green, 1965)], and the radioactivity measured in a liquid-scintillation counter (Beckman model LS 200).

Presentation of results

Some of the results are presented in a graphical form to illustrate more readily the response of the two processes to variations in insulin concentration. The plots presented are representative of at least two similar experiments, and each point represents the mean of at least six incubations with one individual soleus strip in each incubation. All results presented in any Figure represent those obtained in a single experiment performed in one day on one group of animals. In some groups of animals, the rates of glycolysis in muscle are quite different from those obtained in other groups, so that such results are not combined. Nonetheless, the response to insulin concentration is always similar and the concentration that produces half-maximal stimulation of either glycolysis or glycogen synthesis is very reproducible from day to day and from one group of rats to another.

Results and discussion

Insulin is known to increase the rates of membrane transport of glucose, and hence glycolysis, and glycogen synthesis in muscle (see Newsholme & Start, 1973, for review), and the sensitivity of these processes has been investigated in the present work. The rate of glycogen synthesis has been measured by monitoring the incorporation of [U-^{14}C]glucose into glycogen; it has been assumed that the specific radioactivity of glucose 6-phosphate in the muscle rapidly approaches that of glucose in the incubation medium. The rate of glucose transport has been measured indirectly by monitoring the formation of lactate. Glucose transport is usually measured by monitoring glucose uptake calculated from the decrement in glucose concentration in the incubation medium between zero time and the end of the incubation. However, in the present work it was considered important to use approximately physiological concentrations of glucose (5 mM) and, since the amount of tissue is small (about 35 mg), the decrement in glucose concentration was not detectable with any precision. It is assumed that changes in the rate of transport are quantitatively reflected in the rate of formation of lactate; this is expected theoretically, since under these conditions the flux-generating step (pseudo-flux-generating) in glycolysis is glucose transport (Newsholme & Crabtree, 1979) and, of the glucose taken up by the muscle,

the proportions converted into glycogen or oxidized are small (<10% in each case). Preliminary experiments have demonstrated that the stimulation of lactate formation by insulin is the same whether the lactate is measured radiochemically (after separation from glucose and other glycolytic intermediates on an ion-exchange resin) or enzymically (see the Materials and methods section). Furthermore, the maximal stimulation of glycolysis by insulin observed in this work is 2-fold (Fig. 1*a*; Table 1), which is similar to that observed for glucose uptake in the diaphragm and soleus in other work, and that for glycogen synthesis in soleus muscle is 3-fold (Fig. 1*a*; Table 1), which is also similar to previous work on this muscle (Crettaz *et al.*, 1980; Brady *et al.*, 1981).

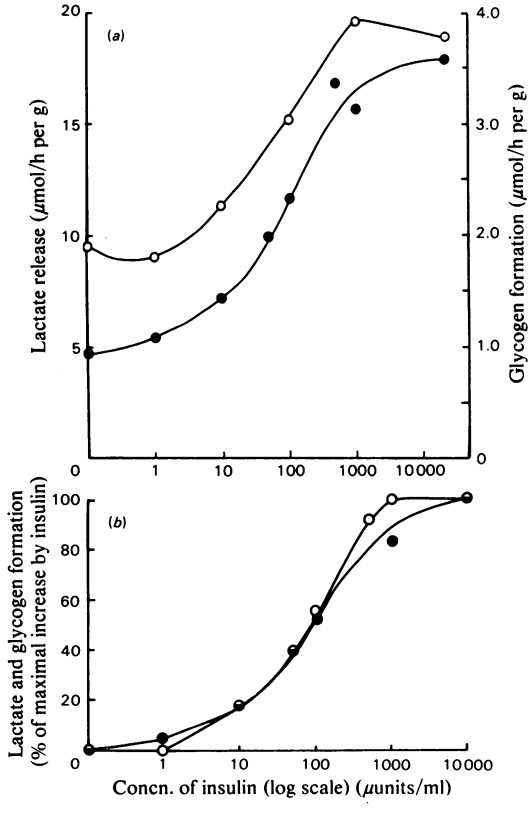

Fig. 1. *Effect of insulin on rates of* (○) *lactate and* (●) *glycogen formation by incubated stripped soleus muscle*
In (*a*) rates of lactate and glycogen formation are presented as μmol/h per g. In (*b*) rates of lactate and glycogen formation are presented as a percentage of the maximal increase by insulin. See the Materials and methods section for details of experimental procedure.

Table 1. *Effect of insulin concentrations on rates of lactate formation and glycogen synthesis in stripped soleus muscle from control (sedentary), exercise-trained and acutely exercised animals*

The methods for measuring rates of lactate formation and glycogen synthesis and for exercising rats are given in the Materials and methods section. Results are presented as means ± S.E.M., with the numbers of separate incubations (each containing one soleus strip) given in parentheses. In some experiments two soleus strips were obtained from one limb; in others only one strip was obtained (depending on the size of the soleus muscle). Statistically significant differences from controls (Student's *t* test) are indicated by $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$.

Concn. of insulin (μunits/ml)	Rates (μmol/h per g of muscle)							
	Lactate formation				Glycogen synthesis			
	Control	Exercise-trained	Control	Acutely exercised	Control	Exercise-trained	Control	Acutely exercised
0	9.73 ± 1.10 (6)	10.36 ± 1.55	8.42 ± 1.15 (6)	7.23 ± 1.09	0.97 ± 0.11 (6)	0.31 ± 0.05***	0.92 ± 0.10 (6)	1.72 ± 0.23**
1	11.78 ± 2.23 (6)	10.01 ± 1.24	7.07 ± 0.75 (6)	6.83 ± 0.34	1.01 ± 0.14 (6)	0.38 ± 0.02***	1.21 ± 0.28 (6)	2.03 ± 0.08**
10	12.24 ± 1.89 (8)	22.36 ± 3.56**	10.81 ± 1.85 (8)	9.52 ± 0.85	1.41 ± 0.15 (8)	0.46 ± 0.03***	1.28 ± 0.14 (8)	1.99 ± 0.17**
100	16.95 ± 2.74 (8)	25.00 ± 3.55**	12.81 ± 1.74 (8)	13.75 ± 1.95	1.89 ± 0.24 (8)	1.36 ± 0.08	2.66 ± 0.33 (8)	3.01 ± 0.32
1000	19.88 ± 1.70 (6)	23.75 ± 3.74*	16.84 ± 3.37 (8)	16.30 ± 1.46	2.36 ± 0.34 (6)	1.66 ± 0.29	3.36 ± 0.48 (8)	3.66 ± 0.34
10000	20.93 ± 3.18 (6)	28.79 ± 3.90*	17.39 ± 1.34 (6)	16.04 ± 1.56	2.22 ± 0.36 (6)	1.56 ± 0.22	3.52 ± 0.52 (6)	3.37 ± 0.19

Insulin-sensitivity in control sedentary animals

The effects of insulin concentration on the rates of glycolysis and glycogen synthesis are shown in Fig. 1(*a*) and Table 1. The sensitivity to insulin is more easily observed from the plots of percentage stimulation of these processes (Fig. 1*b*), which indicate that the half-maximal effect for both processes is observed at about 100 μunits of insulin/ml. In isolated adipocytes, however, the half-maximal effect of insulin on glucose uptake (and lipolysis) is observed at about 10 μunits/ml (Green & Newsholme, 1979) and the difference in the sensitivity between adipose tissue and muscle is clearly demonstrated in Fig. 2. The plasma insulin concentration in starved rats is approx. 4 μunits/ml, and this is increased to about 40 μunits/ml by feeding (Hawkins *et al.*, 1971), and the concentrations at the site of the muscle cell membrane are likely to be lower than these. Hence, if the sensitivity of soleus to insulin is representative of other muscles, and if the sensitivity of adipocytes from the epididymal fat-pad is representative of adipose-tissue depôts *in vivo*, these findings strongly suggest that, in normal sedentary animals, changes in insulin concentration after a meal should have a much larger effect on adipose tissue than on muscle. One effect of insulin on adipose tissue is to decrease the rate of lipolysis, which would decrease the plasma fatty acid concentration and hence increase the rate of glucose utilization by muscle via the glucose/fatty acid cycle. Indeed, the greater sensitivity of adipose tissue to insulin supports the view that this indirect effect of insulin could be of more importance in regulating glucose uptake by muscle than the direct stimulation of glucose transport and glycolysis (see Newsholme, 1977, for discussion of this viewpoint). This leads to the suggestion that, in normal sedentary animals, in order for insulin markedly to increase glucose utilization by muscle, high plasma insulin concentrations would be required.

Insulin-sensitivity immediately after a single period of exercise

Sedentary animals were run for 90min on a treadmill, the soleus muscle was immediately removed and the sensitivity of the muscle was investigated. This single period of exercise did not change either the response of glycolysis or the concentration of insulin that produces the half-maximal effect (Table 1; Fig. 3). The effect of exercise on rates of glycogen synthesis at increasing insulin concentrations is shown in Table 1; the rates of synthesis are increased at the lower concentrations of insulin after exercise, but there is no effect at the higher concentrations. The plot of percentage of maximal increase by insulin against insulin concentration is similar to that in Fig. 3 (results not shown).

Thus after exercise, a greater proportion of the glucose uptake is converted into glycogen at lower insulin concentrations, but there is no increase in the maximal rate. This suggests that glycogen synthesis after exercise may not be markedly influenced by insulin. The effect may be explained by a decrease in the glycogen content of the tissue, since this is known to influence the rate of glycogen synthesis in muscle (Danforth, 1965).

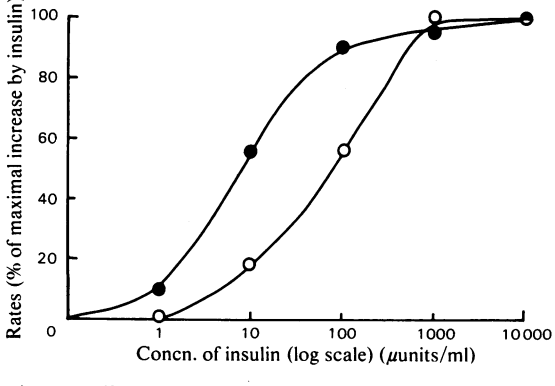

Fig. 2. *Effect of insulin on rate of lactate formation by incubated stripped soleus muscle* (○) *and rate of glucose utilization by isolated adipocytes* (●)

Rates are presented as percentages of the maximal increase by insulin. See the Materials and methods section for details of experimental procedure. Adipocytes were prepared and incubated as described by Green & Newsholme (1979).

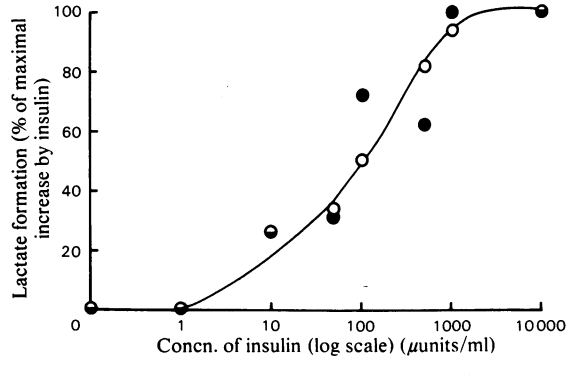

Fig. 3. *Effect of insulin on the rate of lactate formation presented as percentage of the maximal increase in incubated stripped soleus muscle from sedentary* (○) *and exercised* (●) *animals*

Exercised animals ran on a treadmill for 90min and were killed 1h later. See the Materials and methods section for details of experimental procedure.

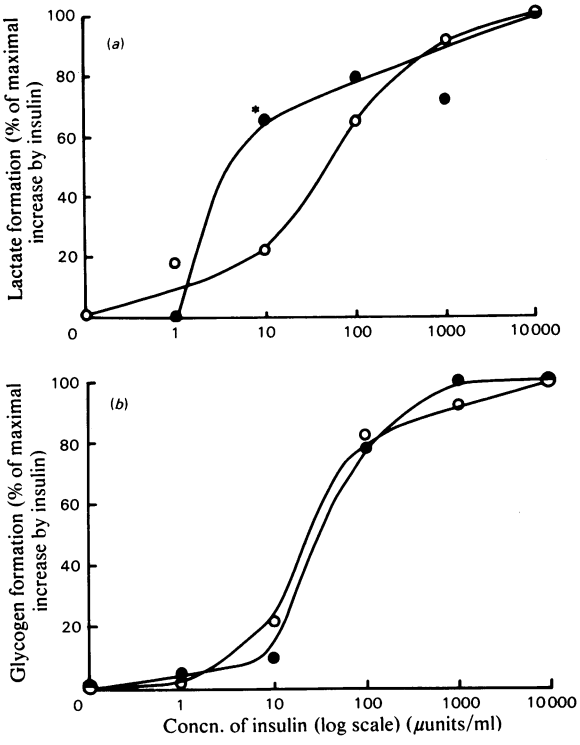

Fig. 4. *Effect of insulin on (a) rate of lactate formation and (b) rate of glycogen formation, presented as percentage of maximal increase in incubated stripped soleus muscle from sedentary* (○) *and exercise-trained* (●) *animals*

Exercise-trained rats were run daily for 6 days a week on a treadmill at 28m/min. See the Materials and methods section for details of experimental procedure. A statistically significant difference from sedentary controls (Student's *t* test) is indicated by $*P < 0.01$.

Exercise-trained animals and insulin-sensitivity

The training regime used in this work resulted in an increase in the maximum activity of hexokinase in the red quadriceps muscle (0.84 to 1.17 μmol/min per g, which was statistically significant), which is similar to the effect of other training regimes (Baldwin & Winder, 1977). In soleus muscle from exercise-trained animals, the maximal response of glycolysis to insulin was increased by about 50% (Table 1), which suggests that the number of carriers for glucose in the cell membrane is increased. Of considerable importance is the finding that the sensitivity of glucose uptake by the soleus to insulin is increased markedly in trained animals; the half-maximal effect was observed at approx. 10 μunits/ml in trained rats, compared with 100 μunits/ml in sedentary controls (Fig. 4*a*). In contrast, there was little or no change in the sensitivity of glycogen synthesis; the concentration of insulin required to produce half-maximal stimulation was between 10 and 100 μunits/ml (Fig. 4*b*). Nonetheless, training did affect the process, since the basal and maximal rates of glycogen synthesis were lower than those in sedentary controls (Table 1). It is noteworthy that in exercise-trained animals the sensitivity of glucose uptake in muscle is similar to that of adipose tissue from sedentary animals (compare data in Figs. 2 and 4*a*). Furthermore, since it has been shown that exercise-training does not affect insulin-sensitivity in adipose tissue (Wardzala *et al.*, 1982), the current findings suggest that in trained animals only low concentrations of insulin are required to stimulate directly glucose utilization in both muscle and adipose tissue.

There is considerable evidence in both man and the rat that training improves glucose tolerance and decreases insulin concentration after oral glucose. The increased insulin-sensitivity of glycolysis in muscle observed in the present work, and also by Richter *et al.* (1982) and Berger *et al.* (1979), could be partly responsible for the findings in the intact animal. Furthermore, impaired glucose tolerance (chemical diabetes) in adult sedentary man can be returned to normal by physical training (Saltin *et al.*, 1979), and this could be explained by the present findings in rats.

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