Glucose clearance in aged trained skeletal muscle during maximal insulin with superimposed exercise

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Dela, F., K. J. Mikines, J. J. Larsen, and H. Galbo.
Glucose clearance in aged trained skeletal muscle during maximal insulin with superimposed exercise. J. Appl. Physiol. 87(6): 2059–2067, 1999.—Insulin and muscle contractions are major stimuli for glucose uptake in skeletal muscle and have in young healthy people been shown to be additive. We studied the effect of superimposed exercise during a maximal insulin stimulus on glucose uptake and clearance in trained (T) (1-legged bicycle training, 30 min/day, 6 days/wk for 10 wk at ~70% of maximal O2 uptake) and untrained (UT) legs of healthy men (H) (n = 6, age 60 ± 2 [SE] yr) and patients with Type 2 diabetes mellitus (DM) (n = 4, age 56 ± 3 yr) during a hyperinsulinemic (~16,000 pmol/l), isoglycemic clamp with a final 30 min of superimposed two-legged exercise at 70% of individual maximal heart rate. With superimposed exercise, leg glucose extraction decreased (P < 0.05), and leg blood flow and leg glucose clearance increased (P < 0.05), compared with hyperinsulinemia alone. During exercise, leg blood flow was similar in both groups of subjects and between T and UT legs, whereas glucose extraction was always higher (P < 0.05) in T compared with UT legs (15.8 ± 1.2 vs. 14.6 ± 1.8 and 11.9 ± 0.8 vs. 8.8 ± 1.8% for H and DM, respectively) and leg glucose clearance was higher in T (H: 73 ± 8, DM: 70 ± 10 ml·min−1·kg−1) compared with UT (H: 63 ± 8, DM: 45 ± 7 ml·min−1·kg−1) but not different between groups (P > 0.05). From these results it can be concluded that, in both diabetic and healthy aged muscle, exercise adds to a maximally insulin-stimulated glucose clearance and that glucose extraction and clearance are both enhanced by training.

Type 2 diabetes mellitus; aging; glycolysis; glycogenolysis; lactate

INSULIN AND CONTRACTIONS (exercise) are the two major stimuli for glucose transport into skeletal muscle, and both are able to recruit GLUT-4 from an intracellular site to the sarcolemma. The two different stimuli may execute their effect alone or in concert, and they have been shown to be additive to one another and to not share the exact same pathway (for a recent review see Ref. 11). These findings are primarily based on studies of rat muscles in vitro. In healthy humans, exercise has been shown to add to the effect of a maximal (1) or submaximal (3) insulin stimulus on whole body glucose disappearance from plasma. In a previous study, we extended the findings in human subjects by demonstrating in young healthy men that the additional effect of exercise on insulin-stimulated glucose uptake is exerted in the contracting muscles (9). Furthermore, we were able to demonstrate that the effect of exercise on maximal insulin-stimulated glucose uptake is higher in trained compared with untrained muscle (9). Normally, during submaximal exercise at a given, absolute workload, glucose uptake in trained muscle will be less than in untrained muscle. This finding occurs because in the trained muscle a higher fraction of the energy expenditure will be covered by fat utilization compared with that in an untrained muscle (13, 15) because of training-induced increases in enzymes involved in β-oxidation and/or a primary reduction in glycogenolysis and glucose transport (14). However, during exercise superimposed on maximal insulin stimulation, the high insulin concentration suppresses lipolysis. The training-induced increase in the capacity of the skeletal muscle to take up glucose will thus be uncovered. This increased capacity in trained muscle reflects increased GLUT-4 content (5, 10), increased capillary density (27), and increased proportion of type I muscle fibers (27) compared with untrained muscle.

Patients with Type 2 diabetes are characterized by insulin resistance in skeletal muscle. Because of the prevailing hyperglycemia, insulin-stimulated glucose uptake rate in skeletal muscle is similar (or often higher) in these patients compared with healthy humans, but the glucose clearance rate is diminished (7). During exercise of the same absolute or relative intensity, leg glucose uptake is higher in lean Type 2 diabetic patients compared with healthy control subjects, probably because of the mass action of the prevailing hyperglycemia. The combined effect of exercise and insulin stimulation on whole body or specifically on leg muscle glucose metabolism has not previously been investigated in either healthy, aged people or in patients with Type 2 diabetes. It follows that the influence of training on these effects has also not been studied before.

In the present study, we investigated the effect of adding submaximal exercise on maximal insulin stimulation in ~60-yr-old healthy men (H) and patients with Type 2 diabetes mellitus (DM). A maximal (supraphysiological) insulin dose was used as the basis for adding the exercise, because in this situation the insulin signaling cascade is fully taxed, and any further effect on glucose uptake in the muscle may be attributed to the exercise. We hypothesized that in both groups exercise would add to the effect of a maximal insulin stimulus. Furthermore, we hypothesized that, in both H and DM, trained muscle in this situation would have
an increased glucose clearance and uptake rate compared with untrained muscle.

MATERIALS AND METHODS

Subjects and Training Regimen

Six H and four DM gave their informed consent to participate in the study, which was approved by the Ethics Committees of Copenhagen and Frederiksberg. All the healthy subjects had normal glucose tolerance (assessed by a 75-g oral glucose tolerance test), and none were taking any medication. Of the patients, three were treated by diet alone and one was treated with both diet and oral antidiabetic drugs (glipizide 3.5 mg twice daily + metformin 500 mg twice daily). On the day of the experiment, no medication was taken. In neither of the groups did the subjects have clinical or laboratory evidence of any other endocrine disease. Subject characteristics are shown in Table 1.

The subjects trained one leg on a modified ergometer bicycle for 10 wk, 6 days/wk, 30 min/day, at an intensity constant relative training intensity. Before and at the end of every training session the subjects wore a heart rate monitor (Polar Electro, Kempele, Finland) and kept a training diary with the workload and the corresponding heart rate. The workload was adjusted upward once a week to ensure a constant relative training intensity. Before and at the end of the training period, maximal oxygen consumption was determined during cycle ergometer exercise tests performed with two legs (V\textsubscript{O\textsubscript{2}}\text{max}) and with each leg separately (peak O\textsubscript{2} consumption).

Experimental Procedure

The subjects were studied in the fasting state. On the experimental day the subjects arrived in the laboratory in the morning and were weighed, and percent body fat was estimated from skinfold measurements (22). The subjects were then placed in bed. Electrocardiogram and heart rate were monitored by precordial electrodes. A catheter was inserted in a medial cubital vein for later infusions of insulin and glucose (20%), and an arterial cannula was inserted in the radial or brachial artery for later sampling of blood and continuous monitoring of blood pressure. In both femoral veins, Teflon catheters were inserted for later sampling of blood and measurements of leg blood flow (thermodilution technique) as previously described (6). One minute before and during every blood sample and blood flow measurement, pneumatic cuffs placed around the subject's ankles were inflated to systolic pressure plus 50 mmHg. Expiratory air was collected in Douglas bags. The subjects were accustomed to the respiratory valve for 4 min before the collection of air (~10 min during rest and ~2 min during exercise).

After basal measurements, a three-step, sequential isoglycemic, hyperinsulinemic clamp was started. Insulin was infused at rates of 28, 88, and 480 m\textsuperscript{U}·min\textsuperscript{−1}·m\textsuperscript{−2} for 120 min each, except for the last step, which lasted 150 min. During the final 30 min of the last clamp step, the subjects performed two-legged bicycle exercise (see details below). Before and after each clamp step and superimposed exercise, the subjects voided, and a urine aliquot was stored at ~20°C. The results obtained at rest have been published previously (7). In the present paper, we only report results obtained at rest (maximal insulin) and during the superimposed exercise from those subjects who completed the superimposed exercise. Thus the data originate from a subgroup of the original eight H and seven DM who participated in the one-legged training program. In one experiment in each group the final 30-min two-legged exercise could not be performed due to technical difficulties with the cycle ergometer, and one H and two DM could not complete the exercise because of physical and mental exhaustion.

The two-legged exercise superimposed on the maximal insulin infusion rate was performed on a rebuilt examination couch that had a bicycle ergometer mounted in one end, allowing the subject to exercise in a semisupine position. The time of the day when this exercise bout was carried out was between 4:00 and 5:30 PM, which was ~24 h after the last training bout. Workloads were adjusted to elicit a heart rate of ~70% of individual maximal heart rate 10 min into the exercise. The pedals of the ergometer were equipped with strain-gauge sensors. Force production was continuously displayed on a recorder, so the subjects could adjust the force used by each leg to apply identical forces with the two legs. Thus the work performed by the trained (T) and the untrained (UT) leg was of the same absolute intensity. After 10 and 25 min of exercise, blood samples and blood flow measurements were obtained, and respiratory air was collected at 20 min. Data on blood flow and release and uptake of substances in the legs are always presented relative to leg weight. Because no difference was detected between measurements done at 10 and 25 min, values from these two time points are given as pooled data.

Calculations and Analytic Procedures

Uptake and release of glucose, alanine, lactate, glycerol, O\textsubscript{2}, and CO\textsubscript{2} were calculated as arteriovenous whole blood concentration difference multiplied by blood flow for the T and UT leg separately. Balance for free fatty acids (FFAs) was calculated as arteriovenous plasma concentration times plasma flow, which is equal to blood flow times (1 – arterial hematocrit). Concentrations of the hormones were measured in arterial plasma. Data on glucose balance across the legs are given as extraction and clearance rates. These were calculated as arteriovenous whole blood glucose concentration difference divided by arterial whole blood glucose concent-

Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Subject</th>
<th>H (n = 6)</th>
<th>DM (n = 4)</th>
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</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>60 ± 2</td>
<td>56 ± 3</td>
</tr>
<tr>
<td>V\textsubscript{O\textsubscript{2}max (2 legged)}, ml·min\textsuperscript{−1}·kg body wt\textsuperscript{−1}</td>
<td>84 ± 4</td>
<td>84 ± 3</td>
</tr>
<tr>
<td>V\textsubscript{O\textsubscript{2peak} (performed with trained leg only)}, ml·min\textsuperscript{−1}·kg body wt\textsuperscript{−1}</td>
<td>29 ± 2</td>
<td>33 ± 1†</td>
</tr>
<tr>
<td>Body mass index, kg/m\textsuperscript{2}</td>
<td>25 ± 1*</td>
<td>25 ± 1*</td>
</tr>
<tr>
<td>Fat mass, %</td>
<td>22 ± 3</td>
<td>22 ± 2</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.6 ± 0.2*</td>
<td>5.7 ± 0.1*</td>
</tr>
<tr>
<td>Fasting insulin, pmol/l</td>
<td>60 ± 6*</td>
<td>54 ± 6*</td>
</tr>
</tbody>
</table>

*Significantly different from DM, P < 0.05. †Significantly different from before training, P < 0.05.
tration multiplied by 100 and expressed in percent (extraction), and as glucose uptake divided by whole blood glucose concentration in the artery (clearance). All blood samples were kept at −20°C until analysis except for samples for FFAs and catecholamines, which were kept at −80°C. Detailed description of stabilization of blood samples and analysis of all hormones, metabolites, and gases have been given previously (4).

Whole body glucose uptake rate was calculated as the steady-state glucose infusion rate averaged over a 10-min period, and whole body glucose clearance rate was calculated as whole body glucose uptake rate divided by the arterial glucose concentration in plasma.

Calculations of whole body glucose oxidation and storage, lipid oxidation, and lipid synthesis were performed on the basis of measurements of O₂ uptake, CO₂ production, and urinary excretion of urea nitrogen and glucose. Protein combustion was calculated as 100/16 times nitrogen excretion corrected for changes in the urea pool. Coefficients for O₂ consumption and CO₂ production in the oxidation of glucose, glycogen, lipids, and protein were as previously published (17). Nonprotein respiratory exchange ratio (RER) was used in all calculations. When nonprotein RER values were <1.0, the only net nonprotein metabolic processes using O₂ and producing CO₂ were assumed to be glucose oxidation and lipid oxidation, whereas when RER >1.0 they were assumed to be glucose oxidation and lipogenesis. Lipogenesis was estimated by assuming that the triglycerides formed were tripalmitate, triesterate, and tripalmitae in the ratio 3:2:1, respectively. Synthesis of this “average” lipid from 1 g of glucose requires 25.8 ml of O₂ and produces 239.6 ml of CO₂. The coefficients of O₂ consumption and CO₂ production were 0.23 and 0.48, respectively. Glucose oxidation in the legs was indirectly determined by the arterial plasma glucose concentration.

Calculations of substrate oxidation and storage in the legs were done on the basis of measurements of O₂ and CO₂ in arterial and venous whole blood, and on the assumption that protein combustion was whole body protein combustion times leg O₂ consumption divided by whole body O₂ consumption. Nonprotein respiratory quotient (RQ) was used in all calculations. Glucose was assumed to be the only nonprotein substrate, and glucose oxidation in the legs was indirectly determined as glucose uptake rate minus glucose oxidation.

Whole body insulin clearance rates were calculated as insulin infusion rate divided by arterial plasma insulin concentration.

Statistics

Results are presented as means ± SE. Differences between groups and effect of training were detected by two-way ANOVA. Differences in parameters that were represented by single measurements were detected by the Student’s t-test. P < 0.05 was considered significant in two-tailed testing in both tests.

RESULTS

In response to the 10-wk one-legged training regimens, body composition changed only in DM, who experienced a slight weight loss (Table 1). V⁰₂max increased (P < 0.05) in both groups (Table 1). Similarly, peak O₂ consumption for the T leg also increased (P < 0.05) in both groups, whereas no change was seen for the UT leg (data not shown). Maximal heart rate did not change in response to training and was always higher (P < 0.05) in H (before: 176 ± 2, after: 171 ± 3 beats/min) compared with DM (before: 164 ± 9, after: 165 ± 2 beats/min). Both fasting plasma insulin and glucose concentrations were lower after training in DM, whereas no change was seen in H (Table 1).

After onset of the two-legged supine bicycling (H: 95 ± 15 W; DM: 88 ± 19 W; P < 0.05), heart rate increased (P < 0.05) and was constant after 10 min of exercise (Fig. 1). The subjects exercised at a heart rate corresponding to 73 ± 2 and 78 ± 2% of individual maximal heart rates in the H and DM, respectively. In response to the exercise, whole body O₂ uptake increased (Table 2) and corresponded to 54 ± 4 and 53 ± 10% of V⁰₂max in the H and DM, respectively (P > 0.05).

Hormonal Responses

During exercise, insulin was infused at the same rate as at rest (480 mU·min⁻¹·kg⁻¹). However, in both groups plasma insulin concentrations increased during exercise (P < 0.05; Fig. 2). If one assumes complete suppression of endogenous insulin secretion, whole body insulin clearance rate decreased from 471 ± 71 and 449 ± 21 ml/min at rest to 389 ± 79 and 381 ± 21 ml/min (both P < 0.05) during superimposed exercise in DM and H, respectively.

Arterial epinephrine and norepinephrine concentrations in plasma increased in response to exercise, with no difference between the groups (Fig. 1). Arterial plasma cortisol and growth hormone concentrations did not change significantly with the superimposed exercise and were not different between the groups (Fig. 1).

Glucose Kinetics

Plasma glucose concentrations were kept constant at the isoglycemic level (i.e., arterial plasma glucose concentrations were maintained at the fasting glycemic level in each subject) also during the superimposed exercise (Fig. 2). When exercise was superimposed on maximal insulin stimulation, whole body glucose uptake and clearance rates always increased (P < 0.05; Fig. 2). In the legs, exercise added significantly to glucose uptake and clearance rates because of marked increases in leg blood flow, whereas the extraction of glucose decreased (Fig. 3).

A positive effect of physical training on leg glucose clearance and uptake was present both during hyperinsulinemia alone and when exercise was added to the hyperinsulinemia in both groups (Fig. 3). During the superimposed exercise, the effect of training was due to a higher fractional glucose extraction in the T compared with the UT leg, whereas leg blood flow during exercise was not influenced by the training status of the leg (Fig. 3).

Both during hyperinsulinemia alone and during exercise, whole body glucose uptake rates were higher (P < 0.05) and whole body glucose clearance rates lower (P < 0.05) in DM compared with H (Fig. 2). In the legs,
With superimposed exercise, leg glucose uptake was still lower in H compared with DM, whereas leg glucose clearance rates were similar in the two groups ($P = 0.26$; Fig. 3).

Fuel Metabolism and Fate of Glucose

Whole body. In response to exercise, oxidation of glycosyl units increased considerably and to the same extent in both groups (Table 2). RER values did not differ between groups, and no change was seen with superimposing exercise (Table 2). Lipid oxidation, which was suppressed during the hyperinsulinemia, increased similarly in both groups when exercise was added (Table 2). Conversion of glucose to lipids could not be detected during either rest or exercise. During insulin alone, the major part of the whole body glucose uptake could be accounted for by glycogenesis ($71 \pm 2$ and $76 \pm 2\%$ in H and DM, respectively ($P > 0.05$), and glycogen synthesis was higher in DM compared with H ($P < 0.05$) (Table 2). When exercise was added, glycogenesis decreased but not to values indicating a net glycogen breakdown (Table 2).

Legs. O$_2$ uptake in the legs in H [2.8 $\pm$ 0.2 and 3.1 $\pm$ 0.2 ml·min$^{-1}$·kg$^{-1}$ for UT and T legs, respectively; not significant (NS)] and in DM (3.2 $\pm$ 0.3 and 4.3 $\pm$ 0.3 ml·min$^{-1}$·kg$^{-1}$ for UT and T legs, respectively; $P < 0.05$) increased when exercise was added to 60 $\pm$ 8 and 60 $\pm$ 5 ml·min$^{-1}$·kg$^{-1}$ for UT and T legs, respectively in H (NS) and to 77 $\pm$ 12 and 82 $\pm$ 16 ml·min$^{-1}$·kg$^{-1}$ for UT and T legs, respectively in DM (NS). There was no difference between O$_2$ uptake in the two groups during either condition.

Table 2. Whole body fuel metabolism and fate of glucose during hyperinsulinemic rest and exercise

<table>
<thead>
<tr>
<th></th>
<th>Insulin</th>
<th>Insulin + Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>V$_{O2}$, ml·min$^{-1}$·kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>3.8 $\pm$ 0.4</td>
<td>16.8 $\pm$ 4.8†</td>
</tr>
<tr>
<td>H</td>
<td>3.3 $\pm$ 0.1</td>
<td>16.7 $\pm$ 1.4†</td>
</tr>
<tr>
<td>Nonprotein RER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>0.96 $\pm$ 0.01</td>
<td>0.95 $\pm$ 0.01</td>
</tr>
<tr>
<td>H</td>
<td>0.93 $\pm$ 0.02</td>
<td>0.95 $\pm$ 0.02</td>
</tr>
<tr>
<td>Glycosyl oxidation, mg·min$^{-1}$·kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>4.1 $\pm$ 0.4</td>
<td>18.4 $\pm$ 3.1†</td>
</tr>
<tr>
<td>H</td>
<td>3.4 $\pm$ 0.2</td>
<td>17.7 $\pm$ 1.6†</td>
</tr>
<tr>
<td>Lipid oxidation, mg·min$^{-1}$·kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>0.2 $\pm$ 0.03</td>
<td>1.5 $\pm$ 0.3†</td>
</tr>
<tr>
<td>H</td>
<td>0.3 $\pm$ 0.06</td>
<td>1.6 $\pm$ 0.6†</td>
</tr>
<tr>
<td>Glycogenesis, mg·min$^{-1}$·kg$^{-1}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DM</td>
<td>13.7 $\pm$ 2.1</td>
<td>4.6 $\pm$ 7.7†</td>
</tr>
<tr>
<td>H</td>
<td>8.6 $\pm$ 0.7*</td>
<td>1.6 $\pm$ 2.1†</td>
</tr>
</tbody>
</table>

Values are means $\pm$ SE. Four DM and 6 H trained 1 leg for 10 wk while keeping the other leg sedentary. They were studied during a hyperinsulinemic clamp at rest and during superimposed 2-legged 30-min exercise at $\Delta V_{O2}$ corresponding to respiration exchange ratio. *Significantly different from DM, $P < 0.05$. †Significantly different from insulin alone, $P < 0.05$. $2062$ INSULIN AND SUPERIMPOSED EXERCISE

Fig. 1. Heart rate (A) and plasma concentrations of various hormones (B: epinephrine; C: norepinephrine; D: cortisol; E: growth hormone) in healthy men (H) ($n = 6$) and in patients with Type 2 diabetes mellitus (DM) ($n = 4$). After 120 min of isoglycemic clamping at $\pm 16,000$ pmol/$l$ of insulin at 0 min, 2-legged semisupine ergometer cycle exercise started and continued for 30 min during the hyperinsulinemia. Values are means $\pm$ SE. *Significant difference between exercise and rest, $P < 0.05$. During hyperinsulinemia alone, glucose uptake was significantly lower in H compared with DM ($P < 0.05$), whereas leg glucose clearance was not significantly different in H compared with DM ($P = 0.07$; Fig. 3).
sulinemia and exercise (UT: 1.03 ± 0.02, T: 1.06 ± 0.02).

With exercise, oxidation of glycosyl units increased in the face of a net glycogen breakdown (Fig. 4). Oxidation of glycosyl units was similar in T and UT legs, but a minor part was due to glycogenolysis in T vs. UT legs (P < 0.05; Fig. 4).

Lactate release was similar in both groups but lower (P < 0.05) in UT compared with T legs during hyperinsulinemia alone. However, when exercise was added to the hyperinsulinemia, release of lactate from UT legs was higher compared with T legs (P < 0.05) in both groups (Fig. 4). Furthermore, during exercise lactate release was increased (P < 0.05) in UT legs (but not in T legs) in DM compared with H (Fig. 4). The percentage of lactate release relative to the overall glycolytic flux (glycosyl oxidation + lactate release + alanine release) was increased (P < 0.05) in UT legs (19 ± 3 and 27 ± 2%, H and DM, respectively) compared with T legs (8 ± 3 and 13 ± 4%, for H and DM, respectively) but was not different between the groups.

In contrast to whole body estimates, lipid oxidation in the legs never differed significantly from zero (data not shown). Furthermore, in line with whole body estimates, lipid synthesis in the legs never differed significantly from zero (data not shown).

FFAs and glycerol were released from the legs before insulin was infused, but no significant release could be detected during hyperinsulinemia with or without exercise (data not shown). Arterial FFA and glycerol concentrations were markedly suppressed during infusion of insulin (Fig. 5). However, when exercise was added, a slight, but significant, increase in glycerol concentrations was seen (Fig. 5).

**DISCUSSION**

In the present study we have shown that, in elderly subjects, whether diabetic or healthy, exercise adds considerably to maximal insulin stimulation in terms of glucose uptake and clearance in whole body (Fig. 2) and skeletal muscle (Fig. 3). Furthermore, we have found that in both groups physical training increases the ability of aged human skeletal muscle to extract glucose and enhance glucose clearance during exercise (Fig. 3). During exercise, glycolysis is increased, and the percentage of lactate release from the T and UT legs to overall glycolytic flux in the legs is similar to previous findings in T and UT legs in young subjects (10). Also in accordance with findings in young subjects (10), when lipolysis is markedly suppressed by insulin, physical training increases glucose oxidation in aged muscle while diminishing glycogen breakdown and lactate production (Fig. 4). The fact that differences in leg glucose clearance between DM and H only approached statistical significance was probably due to the relatively small number of subjects in the DM group. We have previously demonstrated a significant difference by using larger study groups (7).

The present study design was made to evaluate the effect of regular, repeated exercise (i.e., physical training). Therefore, the subjects were studied the day after an exercise bout, which for a trained person is the habitual state. Had we waited further, the subjects would have been studied in a phase of detraining. On the basis of previous studies of whole body metabolism (1, 12, 16, 20, 25, 26) but not on studies using the leg balance technique (8), one might then argue that what is observed in the present study is merely an effect of a
recent, acute bout of exercise. As for the findings during hyperinsulinemia alone, this is proven not to be the case (7). As for the findings during the combined hyperinsulinemia and exercise, it is most unlikely. In a similar study carried out in young, healthy subjects, we have measured the effect of a single exercise bout on the glucose uptake during combined hyperinsulinemia and exercise (9), and we found no difference in glucose uptake rates between a leg subjected to one bout of exercise 24 h earlier and the control leg.

The effect of superimposing exercise on maximal insulin stimulation was a marked increase in glucose clearance (Figs. 2 and 3), and this effect was due to increased blood flow and not fractional glucose extraction, which in fact decreased when exercise was added (Fig. 3). It cannot be excluded that the fractional glucose extraction may have decreased even further because of increases in blood flow per se if training did not increase the glucose transport capacity and diffusion conditions in the skeletal muscles. In the resting hyperinsulinemic situation, the effect of training on glucose clearance was to some extent due to increased blood flow in T vs. UT legs (Fig. 3). In contrast, when exercise was added, blood flow was similar in T vs. UT legs, whereas the fractional glucose extraction was increased in T vs. UT legs, resulting in increased exercise-induced glucose uptake and clearance in T vs. UT legs (Fig. 3). The fact that T legs were able to extract more glucose from the blood compared with UT legs is compatible with previous findings of training-induced increase in skeletal muscle GLUT-4 content (10) and training-induced increased capillary density.

Fig. 3. Leg blood flow (A), glucose extraction (B), glucose clearance (C), and glucose uptake (D) rates in trained (T) and untrained (UT) legs during maximal insulin stimulation, either alone or with superimposed exercise. Values are means ± SE for 6 H (A) and 4 DM (B). ‡Significant difference from trained leg, P < 0.05. †Significant difference from corresponding leg in H, P < 0.05.

Fig. 4. Carbohydrate metabolism during infusion of insulin, either alone or with superimposed exercise. Values are means ± SE given in glucose equivalents per kilogram leg weight for 6 H (A) and 4 DM (B). ‡Significant difference from trained leg, P < 0.05.
in the muscles. In a recent study, during submaximal exercise glucose uptake during normoinsulinemia was lower in T compared with UT human muscle despite higher total GLUT-4 content in T vs. UT muscle, possibly because of less translocation of GLUT-4 to the sarcolemma in trained muscle (24). The reason for the difference between the present study and the study by Richter et al. (24) is most likely due to the fact that the hyperinsulinemia in the present study markedly suppressed lipolysis and thus uncovered the increased glucose transport capacity in T muscle.

During insulin infusion alone, ~93% of whole body glucose uptake could be accounted for by uptake in skeletal muscle in both groups.1 This value is slightly higher than previously reported (88%) (3), but that value was calculated during a submaximal (~700 pmol/l) plasma insulin concentration. During the combined two-legged exercise and hyperinsulinemia, virtually all of the infused glucose could be accounted for by uptake in skeletal muscle. Of the glucose taken up by skeletal muscle, ~30% was accounted for by nonexercising muscle, whereas muscle in UT and T legs accounted for ~30 and ~40%, respectively, in both groups.

For comparisons between T and UT legs during the exercise, it is important that the two legs perform the same absolute amount of work. To this end, the pedals were equipped with strain-gauge sensors, and the force used with each leg during a revolution was displayed to the subject, who therefore easily could adjust his effort with each leg. If such a biofeedback system is not used, the subject will, as he becomes fatigued, perform more work with the T leg to keep up the pedaling frequency. Apart from recordings from the strain-gauge sensors (data not shown), the similar O2 uptake rates in T and UT legs demonstrate that the two legs performed the same absolute amount of work.

In this context, it should be noted that the absolute workload during exercise was not identical in the two groups. Although H were not more fit than DM, their maximal heart rates were higher. An explanation for this may be the presence of autonomic neuropathy in DM. Because the workload was chosen on an individual basis, aiming at 70% of maximal heart rate in each subject after 10 min of exercise, it was necessary to use a higher absolute workload in H. Because V\textsuperscript{O2}\textsubscript{max} did not differ between the two groups, one would have expected that the resulting relative workload was higher in H, but in fact it was calculated to be almost identical (53–54% of V\textsuperscript{O2}\textsubscript{max}) in the two groups. A poor mechanical efficiency and/or less leg muscle mass in DM may be the reason for this discrepancy. It is clear that when the absolute workload was kept similar in T and UT legs, the relative workload was less in T compared with UT legs. During exercise at a lower relative workload, one would expect less carbohydrate utilization (2). However, in the present study exercise was carried out during hyperinsulinemia that inhibited lipolysis and, in turn, fat oxidation. Thus this inhibition unmasked the increased capacity of trained skeletal muscle to utilize glucose during exercise. In this context it is interesting to note that this mechanism was also present in skeletal muscle of DM, a disease that may be regarded as primarily a disease of the skeletal muscle.

DM were studied at their ambient glycemic level (isoglycemic clamp conditions). This was done because insulin-stimulated leg blood flow in this situation is not diminished in Type 2 diabetes mellitus (6). Thus the leg blood flow rates were similar in the two groups at the start of exercise. Had the study been carried out during euglycemia also in DM, leg glucose uptake rates during rest would have been lower compared with those of H. It cannot be excluded than the glucose uptake rates during the superimposed exercise would have been similar in the two groups if the exercise were carried out during euglycemia in both groups, but most likely it would not have been lower in DM compared with H because the contraction-mediated glucose transport is not impaired in Type 2 diabetes mellitus (18).

The rates of glycogenesis during rest and glycogenolysis during exercise were indirectly determined be-

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1 Calculations are done as described in detail by DeFronzo et al. (3).
cause muscle biopsies were not obtained once the insulin infusion had started. However, in the basal state before insulin infusions, muscle glycogen concentrations were 30–40% higher in T compared with UT legs (7), and it is reasonable to expect that this difference was present at the start of exercise as well. A high glycogen concentration will tend to inhibit glucose uptake (23). Nevertheless, glucose uptake rates were higher in T compared with UT legs. Thus the difference in glucose uptake rates would probably have been even greater if glycogen levels had been identical in the two legs. Carbohydrate oxidation rates were similar in T and UT legs (and between groups), whereas glucose uptake rates were higher and lactate release lower in T compared with UT legs. It follows that during exercise glycogenolysis was less in T compared with UT legs. This is an interesting observation because lower rates of glycogen breakdown in trained muscle are usually seen along with increased lipolysis, which was not the case in the present study where lipolysis in both T and UT legs was completely suppressed by the hyperinsulinemia. Thus, in the present study, a link between rate of glycogenolysis and lipolysis, therefore, does not seem to exist.

The lower rates of glycogenolysis in T vs. UT legs during the superimposed exercise may be viewed as either contributing to, or being the consequence of, a training-induced increase in glucose uptake. Low rates of glycogenolysis could contribute to increases in glucose uptake via less inhibition of hexokinase by glucose-6 phosphate, thus facilitating higher glucose uptake rates in T compared with UT legs. On the other hand, increased glucose uptake rates in T legs may essentially be an effect of training-induced increases in GLUT-4 content and capillary density, and lower rates of (net) glycogenolysis the consequence of training-induced increases in glycogen synthase activity (19).

Insulin was infused at the same rate during rest and during exercise. Nevertheless, arterial plasma insulin concentrations increased during exercise (Fig. 1), a phenomenon we have previously seen in young subjects (9). A decrease in insulin clearance because of an exercise-induced reduction in hepatic blood flow probably explains the increase in arterial insulin concentrations.

In summary, we have shown that, in aged human muscle (diabetic or healthy), exercise can add notably to the effect of a maximal insulin stimulus on glucose uptake and clearance. Furthermore, we have shown that, in the face of similar blood flow, exercising trained aged human muscle is capable of extracting more glucose from the blood than is untrained muscle. Finally, in a situation where lipolysis is completely suppressed, glycogen breakdown during exercise is decreased in trained compared with untrained muscle.

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