

# Enhanced Stimulation of Glucose Uptake by Insulin Increases Exercise-Stimulated Glucose Uptake in Skeletal Muscle in Humans

## Studies Using [ $^{15}\text{O}$ ]O<sub>2</sub>, [ $^{15}\text{O}$ ]H<sub>2</sub>O, [ $^{18}\text{F}$ ]fluoro-deoxy-glucose, and Positron Emission Tomography

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In vitro studies have shown that insulin and exercise stimulate glucose uptake in part via distinct mechanisms. We determined whether a high rate of insulin-stimulated glucose uptake (good insulin sensitivity) is associated with an enhanced ability of exercise to increase glucose uptake in vivo in humans. In our study, 22 normal subjects performed one-legged isometric exercise for 105 min (45–150 min) under intravenously maintained euglycemic-hyperinsulinemic conditions (0–150 min). Rates of oxygen consumption, blood flow, and glucose uptake were quantitated simultaneously in skeletal muscle of both legs using [ $^{15}\text{O}$ ]O<sub>2</sub>, [ $^{15}\text{O}$ ]H<sub>2</sub>O, [ $^{18}\text{F}$ ]fluoro-deoxy-glucose, and positron emission tomography. The one-legged exercise, performed at an intensity of 11% of maximal isometric force, was designed to induce similar increases in oxygen consumption in both groups. In the entire group, exercise increased oxygen consumption from  $2.3 \pm 0.3 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$  (insulin) to  $34.2 \pm 3.1 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$  (insulin and exercise) ( $P < 0.001$ ) and muscle glucose uptake from  $60 \pm 6 \mu\text{mol} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$  (insulin) to  $220 \pm 22 \mu\text{mol} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$  (insulin and exercise) ( $P < 0.001$ ). The exercise-induced increase in glucose uptake was due to marked increases in blood flow ( $36 \pm 5 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$  [insulin] vs.  $262 \pm 20 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$  [insulin and exercise],  $P < 0.001$ ) rather than glucose extraction, which decreased from  $2.0 \pm 0.2 \text{ mmol/l}$  (insulin) to  $1.0 \pm 0.1 \text{ mmol/l}$  (insulin and exercise) ( $P < 0.001$ ). The subjects were classified according to their mean rate of whole-body insulin-stimulated glucose uptake into those with high ( $49 \pm 3 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) and normal ( $27 \pm 2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot$

$\text{min}^{-1}$ ) rates of insulin-stimulated glucose uptake. Both insulin-stimulated ( $2.4 \pm 1.1$  vs.  $2.3 \pm 1.2 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$ , normal vs. high insulin sensitivity) and exercise- and insulin-stimulated ( $33 \pm 6$  vs.  $34 \pm 4 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$ ) rates of oxygen consumption were comparable between the groups. Exercise increased glucose uptake more in the group with high insulin sensitivity ( $195 \pm 25 \mu\text{mol} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$ ) than in the group with normal insulin sensitivity ( $125 \pm 19 \mu\text{mol} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$ ) ( $P < 0.05$ ). Muscle blood flow was closely correlated with the rate of oxygen consumption ( $r = 0.91$ ,  $P < 0.0001$ ), and insulin-stimulated ( $30 \pm 5$  vs.  $35 \pm 6 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$ ) and exercise-induced increments ( $222 \pm 31$  vs.  $228 \pm 23 \text{ ml} \cdot \text{kg}^{-1} \text{ muscle} \cdot \text{min}^{-1}$ ) in muscle blood flow were similar between the groups. Glucose extraction remained higher in the group with high insulin sensitivity ( $1.2 \pm 0.2 \text{ mmol/l}$ ) than in the group with normal insulin sensitivity ( $0.7 \pm 0.1 \text{ mmol/l}$ ,  $P < 0.05$ ). We conclude that whereas acute exercise per se increases glucose uptake via increasing glucose delivery, good insulin sensitivity modulates exercise-induced increases in glucose uptake by enhancing cellular glucose extraction. *Diabetes* 49:1084–1091, 2000

**B**oth insulin and contractions stimulate glucose uptake, but the cellular mechanisms appear to be at least in part distinct. Both stimuli translocate GLUT4, the major glucose transporter isoform in skeletal muscle, but possibly from different intracellular pools (1). Insulin uses a phosphatidylinositol (PI) 3-kinase-dependent mechanism, whereas exercise- and contraction-stimulated glucose transport can be blocked by inhibiting calcium release from the sarcoplasmic reticulum (2,3). Recently, it was also demonstrated that contraction-induced increases in glucose uptake do not, in contrast to insulin, involve activation of key insulin signaling mediators such as insulin receptor substrate (IRS)-1 or IRS-2 in skeletal muscle (4). The latter might suggest that good insulin sensitivity (the phrase “insulin sensitivity” is used in a general to describe insulin action), even when due to physical training, does not necessarily increase the ability of exercise to stimulate glu-

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[ $^{18}\text{F}$ ]FDG, [ $^{18}\text{F}$ ]fluoro-deoxy-glucose; AV, arterio-venous; FFA, free fatty acid; IRS, insulin receptor substrate;  $K_t$ , fractional rate of tracer uptake; PET, positron emission tomography; PI, phosphatidylinositol.

cose uptake. On the other hand, insulin and exercise were recently shown to synergistically increase glucose uptake in isolated skeletal muscle, even in mice lacking the insulin receptor in their skeletal muscles (5). The data suggested that insulin action on either non-muscle cells within skeletal muscle or on downstream signaling events enhances the ability of exercise to stimulate glucose uptake.

Previous data addressing the interaction between insulin and exercise on skeletal muscle glucose uptake have demonstrated additive effects of insulin and contractions on glucose uptake in *in vitro* studies in rat epitroclearis muscle (6), perfused rat hindquarter (7), and skeletal muscle membrane vesicles (8). In humans, insulin and exercise have synergistic effects on glucose uptake, i.e., glucose uptake has been greater during combined exercise and insulin stimulation than the sum of exercise- and insulin-stimulated glucose uptakes (9,10). The discrepancy between results *in vitro* and *in vivo* might be explained by the inhibitory effects of free fatty acids (FFAs) on exercise-stimulated glucose uptake under fasting but not hyperinsulinemic conditions and by the enhanced glucose delivery via blood flow *in vivo*. Regarding the impact of interindividual differences in training status or insulin sensitivity on the interaction between exercise and insulin on glucose uptake, data are sparse and controversial. In 2 one-legged training studies, training was found to have no effect on exercise-stimulated glucose uptake (11,12), whereas Dela et al. (13) found greater exercise stimulated glucose uptake in the trained leg than in the untrained leg. Except for these training studies with contrasting results, it is unknown whether good insulin sensitivity enhances the ability of exercise to increase glucose uptake.

Because high insulin sensitivity is associated with increases in  $\dot{V}O_{2\max}$ , capillary density (14), and possibly insulin-stimulated blood flow (15), comparison of the ability of exercise and insulin to stimulate glucose uptake should be performed under conditions in which the exercise stimulus increases oxygen consumption similarly in trained and untrained individuals. Because insulin-sensitive individuals often have higher  $\dot{V}O_{2\max}$  levels than those who are less sensitive, the exercise stimulus should not be chosen based on  $\dot{V}O_{2\max}$  but rather on some other criterion such as strength, which may not differ between the groups. Also, to compare effects of exercise and insulin on cellular (skeletal muscle) glucose uptake, the extracellular milieu should be the same for both stimuli. This result can be achieved by simultaneously quantifying glucose uptake in resting and exercising legs under hyperinsulinemic conditions.

Use of positron emission tomography (PET) combined with radioactive tracers enables noninvasive and direct measurement of regional skeletal muscle metabolism simultaneously in both legs *in vivo* in humans. We applied this methodology to determine 1) whether isometric exercise increases glucose uptake by increasing glucose delivery (blood flow) rather than by increasing glucose extraction and 2) whether high insulin sensitivity of glucose uptake enhances the ability of isometric exercise to increase glucose uptake. For this purpose, a group of normal subjects, divided into those with high insulin sensitivity and those with normal insulin sensitivity based on their mean whole-body glucose uptake rate, were studied under euglycemic-hyperinsulinemic conditions in which one leg was resting and the other leg performed exercise. Intensity was based on maximal isometric strength and

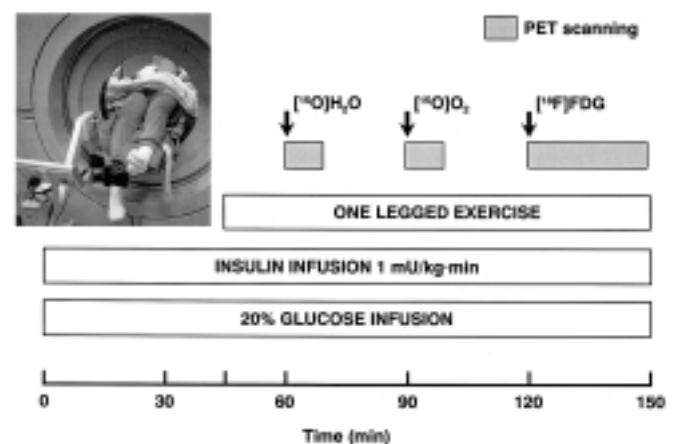
was not different between the groups. Glucose uptake, blood flow, glucose extraction, and oxygen consumption were determined in both legs to determine whether high insulin sensitivity enhances the ability of exercise to stimulate glucose uptake and, if so, whether this can be attributed to glucose delivery or blood flow. The results demonstrate that whereas exercise increases glucose uptake by increasing glucose delivery or extraction, high insulin sensitivity enhances exercise-induced glucose uptake by improving glucose extraction by skeletal muscle.

## RESEARCH DESIGN AND METHODS

**Subjects.** A total of 22 normal male subjects (age  $25 \pm 1$  years, weight  $76 \pm 2$  kg, and BMI  $22.5 \pm 0.7$  kg/m<sup>2</sup>) were recruited for the study. The subjects were healthy, as judged by history, physical examination, and routine laboratory tests. The subjects had no family history of diabetes and used no drugs. The subjects in the normal and high-insulin sensitivity (see RESULTS) groups performed regular aerobic exercise  $2.2 \pm 0.4$  and  $6.4 \pm 2.0$  h/week ( $P = 0.11$ ). Written informed consent was obtained after the purpose, nature, and potential risks were explained to the subjects. The study was approved by the Joint Ethical Commission of the Turku University and Central Hospital.

**Study design.** Before the PET study,  $\dot{V}O_{2\max}$  and maximal isometric contractile force of the quadriceps femoris muscle were determined as detailed below. The design of the PET study is depicted in Fig. 1. The PET study was performed starting between 7:30 and 8:00 A.M. after a 10- to 12-h overnight fast. Two catheters were inserted—one in an antecubital vein for infusion of glucose and insulin and injection of [<sup>15</sup>O]H<sub>2</sub>O and [<sup>18</sup>F]fluoro-deoxy-glucose ([<sup>18</sup>F]FDG) and another in a radial artery for blood sampling. The subjects laid supine during the study, resting between 0 and 45 min, and performed intermittent isometric exercise with one leg between 45 and 150 min, as described in detail below (Fig. 1). At 0 min, a primed continuous insulin infusion ( $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) was started. Normoglycemia was maintained using a variable rate of infusion of 20% glucose (16). The rate of glucose infusion was adjusted based on arterial plasma glucose concentration measurements, which were performed every 5 min. Blood samples were also taken at 30-min intervals for determination of circulating insulin, lactate, FFA, cortisol, growth hormone, noradrenaline, and adrenaline concentrations.

At 60 min, blood flow and at 90 min, skeletal muscle oxygen consumption were measured simultaneously in both legs using [<sup>15</sup>O]H<sub>2</sub>O infusion and [<sup>15</sup>O] inhalation techniques (see below). At 120 min, [<sup>18</sup>F]FDG was injected, and dynamic PET imaging was performed to determine the skeletal muscle glucose uptake (Fig. 1). Blood samples for measurement of radioactivities were taken



**FIG. 1.** Study design. Insulin was infused intravenously at a rate of  $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , and glucose was infused intravenously to maintain normoglycemia. The insert shows the setup for the one-legged exercise, which consisted of intermittent isometric contractions at an intensity that represented 11% of the subjects' maximal isometric force. [<sup>15</sup>O]H<sub>2</sub>O (intravenous bolus), [<sup>15</sup>O]O<sub>2</sub> (inhalation), and [<sup>18</sup>F]FDG (intravenous bolus) were administered to quantify rates of blood flow, oxygen consumption, and glucose uptake simultaneously in both legs.

as detailed below. Blood pressure and heart rate were monitored with an automatic oscillometric blood pressure monitor (HEM-705C; Omron, Tokyo). **Measurement of blood flow, oxygen consumption, and glucose uptake with PET techniques**

**Production of PET tracers.** For production of [<sup>15</sup>O] compounds (radioactive half-time 2 min), a low-energy deuteron accelerator Cyclone 3 was used (Ion Beam Application, Louvain-la-Neuve, Belgium). [<sup>15</sup>O] was produced by the [<sup>14</sup>N](d,n)[<sup>15</sup>O] reaction on natural nitrogen gas (17). Radiochemical purity of [<sup>15</sup>O]O<sub>2</sub> was 97%. [<sup>15</sup>O]H<sub>2</sub>O was produced using a dialysis technique in a continuously working water module (18). Sterility and pyrogen tests were performed daily to verify the purity of the product. [<sup>18</sup>F]FDG (radioactive half-time 109 min) was synthesized with an automatic apparatus as described by Hamacher et al. (19). The specific radioactivity at the end of the synthesis was 76 GBq/μmol, and the radiochemical purity exceeded 98%.

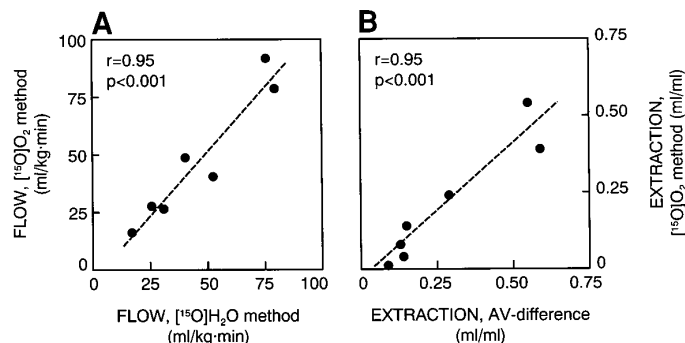
**Image acquisition and processing.** An 8-ring ECAT 931/08-12 tomograph was used (Siemens/CTI, Knoxville, TN). The scanner had an axial resolution of 6.7 mm and in-plane resolution of 6.5 mm. The images were obtained from the femoral region. To correct for photon attenuation, a transmission scan was performed for 20 min with a removable ring source containing <sup>68</sup>Ge. For measurement of blood flow, 1.1–1.7 GBq of [<sup>15</sup>O]H<sub>2</sub>O was injected intravenously, and dynamic scanning was started for 6 min (6 × 5, 6 × 15, and 8 × 30 s). To determine the input function, arterial blood was continuously withdrawn with a pump at a speed of 6 ml/min from the radial artery, and the radioactivity concentration was measured using a 2-channel detector system calibrated to the well counter and PET scanner, as previously described (20). At 90 min, measurement of muscle oxygen consumption was started and [<sup>15</sup>O]O<sub>2</sub> was pumped into a rubber bag and mixed with air. Thereafter, the subjects' nostrils were closed and they inhaled the gas containing 1.38 ± 0.1 GBq via a mouthpiece and a short connecting ventilator hose for 1 min. PET imaging of the femoral region was thereafter performed for 7 min with time frames of 6 × 5, 6 × 15, 6 × 30, and 2 × 60 s. Eight activity samples were collected every 30 s after the 1-min inhalation. For measurement of glucose uptake, 0.15–0.22 GBq of [<sup>18</sup>F]FDG was injected intravenously over 2 min, dynamic scanning for 30 min (8 × 15, 2 × 30, 2 × 120, 1 × 180, and 4 × 300 s) was started, and blood samples for measurement of plasma radioactivity were withdrawn, as previously described (21). All data were corrected for dead time, decay, and measured photon attenuation and reconstructed with a new iterative reconstruction algorithm using median root prior, which improves image quality by reducing the spatial noise without blurring the edges of the object (22).

**Measurement of oxygen consumption in skeletal muscle.** Measurement of muscle oxygen consumption is based on the principle of inert gas exchange between blood and tissue (23). Models using a bolus inhalation have been widely used for measurement of oxygen consumption in the brain (24,25). The relationship between regional oxygen extraction fraction (rOEF), arterial oxygen concentration ([O<sub>2</sub>]<sub>a</sub>), blood flow (rMBF), and regional tissue oxygen uptake (rMRO<sub>2</sub>) is as follows (25):

$$rMRO_2 = [O_2]_a \times rOEF \times rMBF$$

The parameters rOEF, rMBF, and rMRO<sub>2</sub> were quantitated with nonlinear fitting from the [<sup>15</sup>O]O<sub>2</sub> data and by including a separate compartment for free and oxygen-bound myoglobin fitting (25,26). To compare [<sup>15</sup>O]O<sub>2</sub>-derived regional O<sub>2</sub> uptake rates with those obtained using the Fick principle, we measured femoral blood flow using H<sub>2</sub>O (20,27) and arterio-venous (AV) differences of O<sub>2</sub> (Ciba Corning Automatic Blood Gas Analyzer; Ciba-Corning Diagnostics, Medfield, MA) and femoral O<sub>2</sub> uptake using PET and the [<sup>15</sup>O]O<sub>2</sub> bolus inhalation technique. Seven such validation studies were performed in normal subjects either during resting conditions or exercise. The exercise protocol was similar to that used in the present study. Leg blood flow was measured with [<sup>15</sup>O]H<sub>2</sub>O immediately before the [<sup>15</sup>O]O<sub>2</sub> inhalation, and AV samples were collected at 0, 5, and 10 min after the beginning inhalation. The correlation coefficient between all oxygen uptake measurements performed with PET and [<sup>15</sup>O]O<sub>2</sub> and with AV differences and [<sup>15</sup>O]H<sub>2</sub>O was 0.95 ( $P = 0.0012$ ; Fig. 2). Blood flow rates measured with [<sup>15</sup>O]O<sub>2</sub> correlated with those measured with [<sup>15</sup>O]H<sub>2</sub>O ( $r = 0.95$ ,  $P = 0.001$ ; Fig. 2). The reproducibility of oxygen consumption measurements performed with PET and [<sup>15</sup>O]O<sub>2</sub> under resting ( $n = 3$ ) and exercise ( $n = 7$ ) conditions was performed in 10 subjects. The coefficient of variation of 2 repeated measurements performed during the same study was 9.1 ± 1.6%.

**Calculation of blood flow.** The methods to measure blood flow with [<sup>15</sup>O]H<sub>2</sub>O and PET are based on the principle of inert gas exchange between blood and tissues developed by Kety (23). We used the autoradiographic method to calculate the blood flow pixel by pixel using a 200-s integration time and an arterial input curve corrected for dispersion and delay, as previously described (20,27). The method to measure blood flow in muscle using the



**FIG. 2. Comparison of rates of blood flow measured using [<sup>15</sup>O]H<sub>2</sub>O- and [<sup>15</sup>O]O<sub>2</sub>-based PET methods (A) and rates of oxygen consumption measured from AV oxygen concentration differences and the [<sup>15</sup>O]O<sub>2</sub> and PET method (B).**

autoradiographic method, [<sup>15</sup>O]H<sub>2</sub>O, and PET has been validated against the steady-state method (20) and against blood flow measured with strain-gauge plethysmography (27). The reproducibility of repeated flow measurements under resting ( $n = 15$ ) and exercise ( $n = 5$ ) conditions was determined in 20 normal subjects. The coefficient of variation of 2 repeated measurements performed during the same study was 7.2 ± 1.2%.

**Calculation of regional glucose uptake.** The 3-compartment model of [<sup>18</sup>F]FDG kinetics and graphical analysis according to Patlak and Blasberg (28) were used, as previously described (21). Plasma and tissue time-activity curves were analyzed graphically to quantitate the fractional rate of tracer uptake  $K_1$  (28). A minimum of 6 points were used to determine the slope by linear regression. The rate of the glucose uptake (rGU) is obtained by multiplying  $K_1$  by the plasma glucose concentration [Glc]<sub>p</sub> divided by a lumped constant term (LC):  $rGU = [Glc]_p / LC \times K_1$ . The lumped constant accounts for differences in the transport and phosphorylation of [<sup>18</sup>F]FDG and glucose. A lumped constant value of 1.0 for skeletal muscle was used, as previously described (21), although recent studies using 3 independent techniques have suggested the lumped constant term to be slightly >1 (1.1–1.2) (29–31). Glucose extraction was calculated using the Fick equation by dividing muscle glucose uptake by muscle blood flow (27). Femoral muscle glucose uptake measured with [<sup>18</sup>F]FDG and PET is closely correlated ( $r = 0.74$ – $0.87$  [21,32,33]) with whole-body glucose uptake during hyperinsulinemia at low and high FFA concentrations (21) and in normal subjects and competitive runners (32), men and women (33), and hypertensive subjects (34). The reproducibility of glucose uptake measurements using [<sup>18</sup>F]FDG and PET is 12.9 ± 2.1% (27).

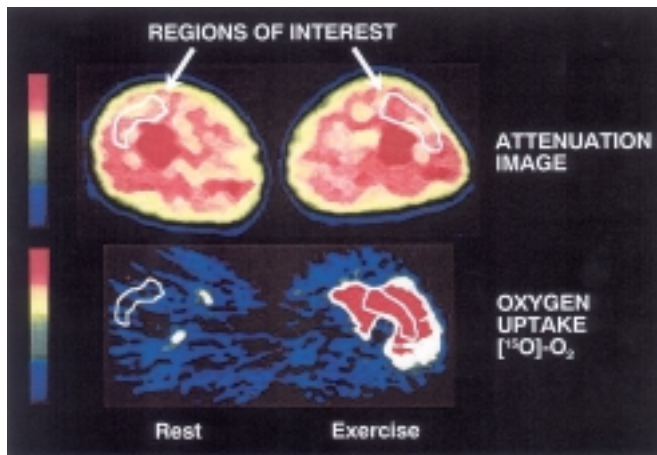
**Regions of interest.** Regions of interest were drawn in the anteromedial muscle compartments of both femoral regions in 4 consecutive cross-sectional slices, carefully avoiding large blood vessels. Localization of the muscle compartments was verified by comparison of the flow images with the transmission image, which provided a topographical distribution of tissue density. The regions of interest outlined in the flow images were copied to the [<sup>15</sup>O]oxygen and [<sup>18</sup>F]FDG radioactivity images to obtain quantitative data from identical regions (Figs. 3 and 4).

**Whole-body glucose uptake.** Whole-body glucose uptake was determined independent of the PET measurements using the euglycemic-hyperinsulinemic clamp technique, as previously described (16). During hyperinsulinemia, normoglycemia was maintained using a variable rate infusion of 20% glucose based on arterial plasma glucose measurements (16). The rate of glucose uptake was calculated during the time period when the measurements of blood flow, oxygen consumption, and muscle glucose uptake were performed (60–150 min). The reproducibility of whole-body glucose uptake measurements in normal men studied twice on separate days is 5 ± 1% (35).

**Measurement of maximal isometric knee extension force, design of exercise during the PET study, and measurement of maximal oxygen uptake**

**Maximal isometric force.** Maximal isometric force of the knee extensors was measured before the PET study with a dynamometer (KinCom; ChatteX, Chattanooga, TN).

**Exercise during the PET study.** The subjects were lying supine in the PET scanner with the femoral regions in the gantry, and the right leg, fixed at an angle of 50°, was fastened to a dynamometer (I-KON; Chattanooga Group, Oxfordshire, U.K.) (Fig. 1). Exercise consisted of 2 s of isometric knee extension intermittent with 2 s of rest during 45–150 min of hyperinsulinemia. Exercise intensity was set at 11% of maximal isometric force because preliminary studies showed it to be feasible to maintain this intensity for the entire study period.

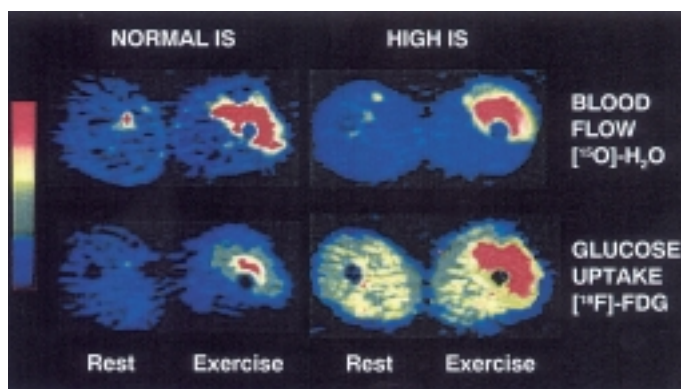


**FIG. 3.** Cross-sections of the femoral region during euglycemic-hyperinsulinemic conditions in which one leg is resting (Rest) and the other exercising, as described in RESEARCH DESIGN AND METHODS and Fig. 1. The example (top panel) denotes the regions of interest, which were outlined for quantification of oxygen consumption, blood flow, and glucose uptake. The lower panel illustrates oxygen consumption as visualized during PET scanning.

The subject performed isometric exercise after a sound signal. The intensity of the exercise was monitored by a light signal, which was green if the intensity of the exercise corresponded to 11% of maximal isometric force (Fig. 1).  $\dot{V}O_{2max}$ .  $\dot{V}O_{2max}$  was determined by cycle ergometry. The criteria used to establish the  $\dot{V}O_{2max}$  were a plateau in  $\dot{V}O_2$  despite increasing intensity and a respiratory quotient >1.1.

**Analytical procedures.** Plasma glucose was determined in duplicate by the glucose oxidase method using an Analox GM7 Glucose Analyzer (Analox Instruments, London, U.K.). Serum insulin concentrations were measured by immunoassay (36), serum FFAs (37) by a fluorometric method, and lactate (38) by a spectrophotometric method. Serum growth hormone was measured using a solid-phase 2-site immunoradiometric assay kit (ELISA-HGH; CIS Bio International, Cedex, France) and serum cortisol by radioimmunoassay (Cortisol [ $^{125}I$ ] Radioimmunoassay; Orion Diagnostica, Espoo, Finland). Plasma catecholamines were measured by high-pressure liquid chromatography and electrochemical detection (39).

**Statistical analysis.** All results are expressed as mean  $\pm$  SE. The differences between the 2 groups were compared using Student's unpaired *t* test where appropriate. Concentrations of hormones and metabolites over time between the 2 groups were analyzed using analysis of variance for repeated measures followed by pair-wise comparison using the Tukey's studentized range test. Pearson's correlation coefficients were calculated where appropriate. Statistical calculations were performed using the SAS statistical program package (SAS Institute, Gary, NC).



**FIG. 4.** Examples of individuals with normal and high insulin sensitivity (IS) under euglycemic-hyperinsulinemic conditions, as in Fig. 3, but during measurement of blood flow and glucose uptake.

## RESULTS

**Metabolic characteristics.** Characteristics of the subjects, divided into those with normal and those with high sensitivity based on their mean rate whole-body insulin sensitivity, are shown in Table 1. The subgroups differed with respect to  $\dot{V}O_{2max}$  and fasting serum insulin concentrations but not with respect to age, fasting glucose concentrations, BMI, or maximal force of knee extensors. Workloads during exercise were similar in the 2 groups (Table 1). The individuals with high insulin sensitivity exercised  $6.4 \pm 2.0$  h/week and those with normal insulin sensitivity  $2.2 \pm 0.4$  h/week ( $P = 0.11$ ).

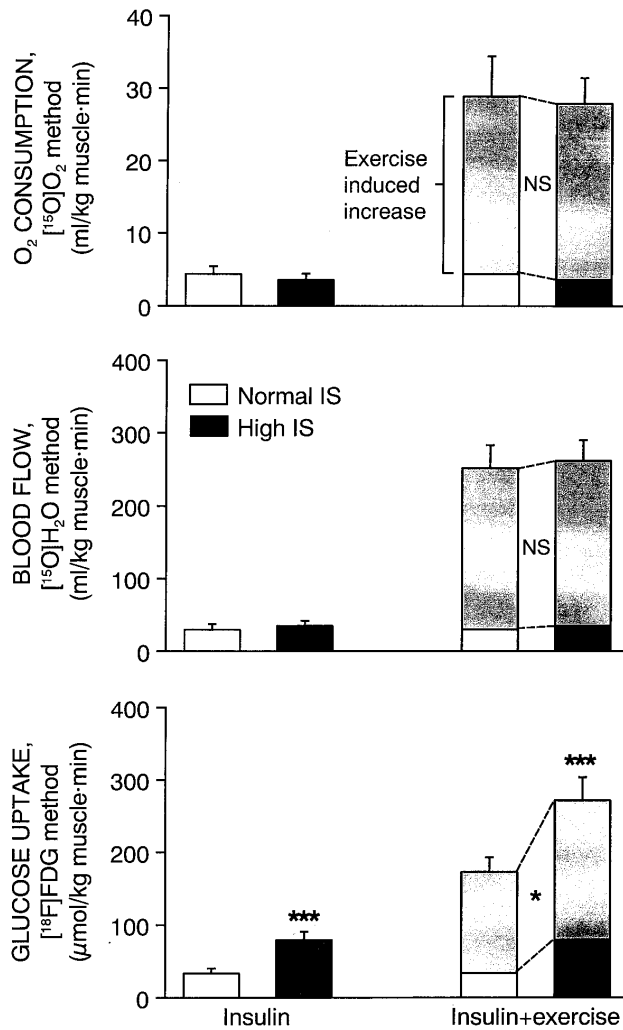
**Oxygen consumption and blood flow.** Resting rates of oxygen consumption were comparable during hyperinsulinemia between the subjects with normal ( $2.4 \pm 1.1$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ ) and high ( $2.3 \pm 1.2$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ ) insulin sensitivity. During exercise and insulin stimulation, rates of oxygen consumption ( $33 \pm 6$  and  $34 \pm 4$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ , respectively) were 14-fold higher than those during hyperinsulinemia alone and were not different between the groups (Figs. 3 and 5). The exercise-induced increases in oxygen consumption ( $30 \pm 6$  and  $32 \pm 4$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ , respectively) were also comparable (Figs. 3 and 5). During hyperinsulinemia, oxygen extraction ratios averaged  $0.40 \pm 0.07$  in subjects with normal insulin sensitivity and  $0.47 \pm 0.09$  in subjects with high insulin sensitivity. During exercise and hyperinsulinemia, these ratios averaged  $0.59 \pm 0.06$  and  $0.64 \pm 0.05$ , respectively (NS vs. hyperinsulinemia for both groups).

In the entire group, exercise increased muscle blood flow from  $36 \pm 5$  to  $262 \pm 20$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$  ( $P < 0.001$ ). Resting rates of muscle blood flow were similar during hyperinsulinemia between the subjects with normal ( $30 \pm 5$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ ) and high ( $35 \pm 6$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ ) insulin sensitivity. During exercise and insulin stimulation, muscle blood flow ( $247 \pm 28$  and  $262 \pm 27$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ , respectively) increased 8-fold, with no difference between the groups (Fig. 4). The exercise-induced increases in oxygen consumption ( $22 \pm 3$  vs.  $23 \pm 2$  ml  $\cdot$  kg $^{-1}$  muscle  $\cdot$  min $^{-1}$ ) were also comparable.

**TABLE 1**  
Subject characteristics

	Normal insulin sensitivity	High insulin sensitivity
<i>n</i>	11	11
Age (years)	25 $\pm$ 1	25 $\pm$ 1
Weight (kg)	75 $\pm$ 2	75 $\pm$ 2
BMI (kg/m $^2$ )	22.2 $\pm$ 0.6	22.2 $\pm$ 0.5
$\dot{V}O_{2max}$ (ml $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ )	40 $\pm$ 2	54 $\pm$ 3§
Maximal isometric force (N)*	645 $\pm$ 56	556 $\pm$ 39
Work force during exercise		
Absolute (N)	73 $\pm$ 8	68 $\pm$ 7
Percentage of maximal	11.4 $\pm$ 0.1	11.3 $\pm$ 0.1
Fasting plasma glucose (mmol/l)	5.3 $\pm$ 0.1	5.2 $\pm$ 0.1
Fasting serum insulin (mU/l)	7.3 $\pm$ 0.8‡	4.0 $\pm$ 0.6
Whole-body glucose uptake ( $\mu$ mol $\cdot$ kg $^{-1}$ $\cdot$ min $^{-1}$ )†	27 $\pm$ 2	49 $\pm$ 3§

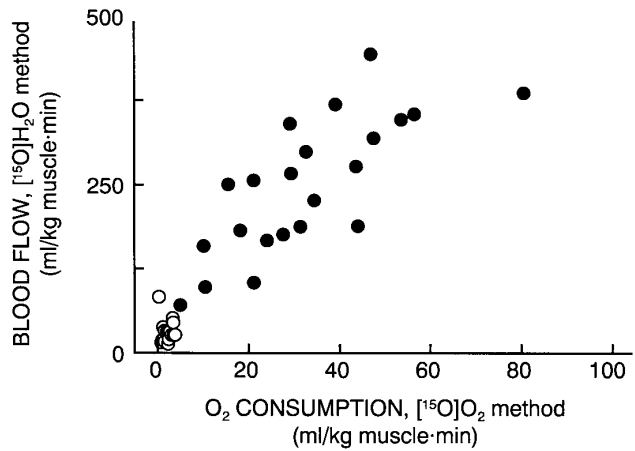
\*Maximal isometric force of knee extensors. †Calculated during 60–150 min. ‡ $P < 0.01$ ; § $P < 0.001$  for normal vs. high insulin sensitivity.



**FIG. 5.** Rates of skeletal muscle oxygen consumption (A), blood flow (B), and glucose uptake (C) during hyperinsulinemia (Insulin) and exercise and hyperinsulinemia (Insulin + exercise) in subjects with normal and high insulin sensitivity (IS). The cross-hatched bars denote the exercise-induced increments in oxygen consumption, blood flow, and glucose uptake. \**P* < 0.05, \*\*\**P* < 0.001 for normal vs. high IS.

min<sup>-1</sup>, respectively) were also comparable between the groups (Fig. 4). Muscle blood flow (measured with [<sup>15</sup>O]H<sub>2</sub>O) was significantly correlated with oxygen consumption (*r* = 0.91, *P* < 0.0001; Fig. 6). This correlation coefficient was 0.21 (NS) in the resting and 0.72 (*P* = 0.0002) in the exercising leg during hyperinsulinemia.

**Glucose uptake.** During hyperinsulinemia, glucose uptake in resting femoral muscle of the subjects with high whole-body insulin sensitivity was 112% higher (81 ± 10 μmol · kg<sup>-1</sup> body wt · min<sup>-1</sup>) than in subjects with normal insulin sensitivity (38 ± 4 μmol · kg<sup>-1</sup> body wt · min<sup>-1</sup>, *P* < 0.005; Fig. 5). The correlation coefficient between glucose uptake in femoral muscle and the whole body was 0.75 (*P* < 0.001). In the leg exposed both to insulin and exercise, glucose uptake increased by 195 ± 25 μmol · kg<sup>-1</sup> muscle · min<sup>-1</sup> in the group with high insulin sensitivity. This result was significantly higher than that in the group with normal insulin sensitivity, in which glucose uptake increased with



**FIG. 6.** The relationship between oxygen consumption and blood flow during hyperinsulinemia (○, insulin) and hyperinsulinemia and exercise (●, insulin + exercise).

exercise by 125 ± 19 μmol · kg<sup>-1</sup> muscle · min<sup>-1</sup> (*P* < 0.05; Fig. 5). Insulin-stimulated glucose uptake in resting femoral muscle was significantly correlated with the exercise-induced increase (*r* = 0.45, *P* = 0.035). The difference in the increase in glucose uptake by exercise between the groups remained significant, even after adjusting for interindividual variation in the increase in oxygen consumption induced by exercise (*r* = 0.49, *P* = 0.030 for group effect in analysis of variance using the increment in oxygen consumption as a covariate).

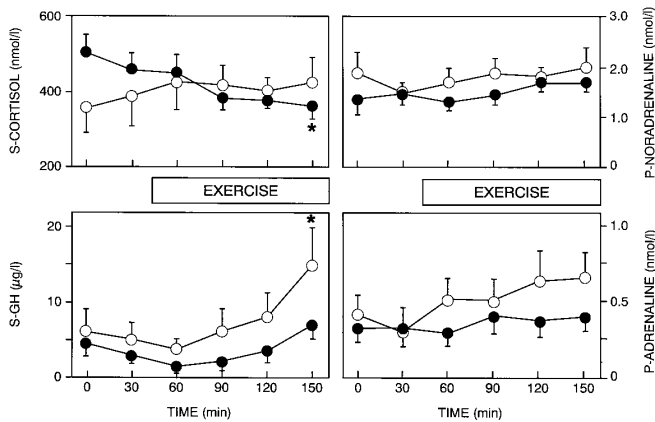
**Glucose extraction.** The fraction of glucose extracted during insulin stimulation in resting muscle was significantly higher in the group with high (0.47 ± 0.07) versus normal (0.27 ± 0.03, *P* < 0.02) insulin sensitivity. Exercise resulted in a significant and an ~50% decrease in fractional glucose extraction in both groups (-0.25 ± 0.06 vs. -0.13 ± 0.03 in groups with high vs. normal insulin sensitivity, NS between groups). In the leg exposed to both insulin and exercise, fractional glucose extraction was significantly higher in the group with high (0.24 ± 0.04) versus normal (0.13 ± 0.02, *P* < 0.05) insulin sensitivity.

**Counterregulatory hormone and plasma lactate concentrations.** During the 45-min period before exercise, circulating concentrations of cortisol, growth hormone, adrenaline, and noradrenaline remained unchanged (Fig. 7). During the period of exercise (45–150 min), serum growth hormone and plasma adrenaline concentrations increased slightly in the group with normal insulin sensitivity (*P* < 0.05 for 150 vs. 0 min; Fig. 7). The changes between the groups were not significantly different. Plasma lactate concentrations increased slightly over time (*P* < 0.05) in the entire group (plasma lactate 0.87 ± 0.08 mmol/l at 0 min, 1.1 ± 0.1 mmol/l at 30 min, 1.1 ± 0.1 mmol/l at 60 min, 1.0 ± 0.1 mmol/l at 90 min, 1.1 ± 0.1 mmol/l at 120 min, and 0.9 ± 0.1 mmol/l at 150 min) with no difference between the groups at any time point (data not shown).

**DISCUSSION**

Studies *in vitro* have demonstrated that the mechanisms by which insulin and exercise stimulate glucose uptake have both similarities and differences (1,40). Such data raise the possibility that variation in insulin sensitivity may or may not influence exercise-induced increases in glucose uptake. In the present





**FIG. 7. Serum concentrations of counterregulatory hormone during the hyperinsulinemic (0–150 min) and exercise (45–150 min) periods in the subjects with normal (○) and high (●) insulin sensitivity (IS). \* $P < 0.05$  for change vs. 0 min in the normal insulin sensitivity group. P, plasma; S, serum; S-GH, serum growth hormone.**

study, we determined whether high insulin sensitivity is associated with an enhancement in exercise-stimulated glucose uptake in skeletal muscle *in vivo* in humans. The experimental design was such that any difference in exercise-induced glucose uptake between the experimental groups would not be attributed to differences in oxygen consumption or in extracellular counterregulatory hormone or metabolite concentrations. To achieve this result, effects of insulin and exercise on glucose uptake were compared by simultaneously quantitating glucose uptake in resting and exercising legs under intravenously maintained euglycemic-hyperinsulinemic conditions (Fig. 1). When the exercise stimulus was superimposed on insulin, glucose uptake, blood flow, and oxygen consumption increased severalfold. These changes were contrasted with a highly significant decrease in glucose extraction. These data confirm previous data by DeFronzo et al. (9) and Dela et al. (13), who quantitated effects of dynamic bicycle exercise in the entire leg using catheterization techniques and showed that exercise increases glucose uptake via increases in glucose delivery under insulin-stimulated conditions. Our present data extend these findings by demonstrating that acute exercise, also of the intermittent isometric type and when measured using PET techniques directly in skeletal muscle, increases glucose uptake by enhancing glucose delivery. This result occurs because acute exercise increases oxygen consumption and blood flow, whereas glucose extraction decreases oxygen consumption and blood flow. In addition, to our knowledge, we compared for the first time the ability of isometric exercise to increase glucose uptake in groups with different insulin sensitivities. This comparison revealed that the group with high insulin sensitivity had a significantly higher exercise-induced increase in glucose uptake than the group with normal insulin sensitivity. In contrast to the mechanism underlying acute exercise-stimulated glucose uptake *per se*, insulin sensitivity appeared to enhance exercise-stimulated glucose uptake by enhancing the ability of muscle cells to extract glucose from the circulation. Thus, although the pathways by which exercise and insulin stimulate glucose uptake are characterized by distinct differences *in vitro*, there are similarities that result in greater stimulation of glucose uptake by exercise in subjects with high insulin sensitivity compared with subjects with nor-

mal insulin sensitivity. Recent data demonstrating synergism of insulin and exercise on glucose transport in skeletal muscle from mice lacking the insulin receptor in muscles (5) suggest that such similarities in insulin- and exercise-stimulated signaling pathways could involve greater stimulation of insulin postreceptor pathways in muscle fibers or greater effects of insulin on non-muscle cells within skeletal muscle in subjects with high compared with normal insulin sensitivity.

Use of PET for quantification of skeletal muscle perfusion and metabolism offers some advantages over previous techniques. This technique allows direct assessment of skeletal muscle perfusion and metabolism without interference from other leg tissues and without invasive catheterizations. Furthermore, PET allows quantification of metabolism within regions of skeletal muscle. In the present study, this method allowed quantification within the portion of femoral muscle in which exercise increased oxygen consumption (Fig. 3). We quantified skeletal muscle oxygen consumption using [ $^{15}\text{O}$ ]-labeled oxygen and PET. This method has previously been validated for measurement of oxygen consumption in the brain (24,25). We now use this method to determine oxygen consumption in resting and exercising skeletal muscle. The validation study comparing oxygen extraction measured using AV oxygen concentration differences and [ $^{15}\text{O}$ ]O<sub>2</sub> showed the 2 methods to be closely correlated. The finding of a highly significant relationship between oxygen consumption and blood flow in the exercising leg makes physiological sense and also supports validity of the methodology. Of note, the AV differences reflect oxygen consumption in the entire leg and not locally in the femoral region like the PET measurements. On the other hand, rates of resting blood flow and oxygen consumption were not significantly correlated. Whether this is a true phenomenon or is caused by relatively higher noise from background radioactivity under resting compared with exercise-stimulated conditions cannot be determined with certainty.

In the resting leg exposed to insulin, glucose extraction rather than blood flow distinguished the 2 groups with different insulin sensitivity. This result may seem surprising in view of an earlier study by Hardin et al. (41), who found a greater increase in blood flow by insulin in trained versus untrained subjects. However, this apparent discrepancy is likely to be explained by the difference in the insulin dose used. We infused insulin at a rate ( $1 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ ) that resulted in insulin concentrations comparable to those observed postprandially (42). These concentrations increased glucose extraction 10-fold but had only small effects on blood flow (43). Our study design differs from that of Hardin et al. (41), who infused insulin at a rate of  $15 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ , which does markedly increase blood flow even in untrained subjects (44). Regarding the exercising leg, blood flows were also comparable between the 2 groups under these conditions in the present study. Because the insulin-sensitive group had a higher rate of maximal oxygen consumption, a dynamic exercise stimulus would unlikely have allowed matching of both the absolute and relative workloads. This was why exercise intensity was based on force rather than oxygen consumption. Because there was no difference in muscular strengths between the groups (Table 1), similar absolute and relative workloads could be used, and rates of oxygen consumption were matched. Therefore, the similarity in blood flows in the exercising leg between the groups was expected and sup-

ported experimentally by the finding of a significant correlation between oxygen consumption and blood flow in skeletal muscle. Of note, this result is at variance with that of DeFronzo et al. (9), who observed a significant correlation between glucose uptake and blood flow during exercise, possibly because in this study, the subjects performed bicycle exercise at 40% of their  $\dot{V}O_{2\max}$ . Such design will induce higher oxygen consumption rates in trained versus untrained subjects, although oxygen consumption was not measured in the latter study (9).

Regarding causes of enhanced glucose extraction during exercise in subjects with high versus normal insulin sensitivity, one should consider the possibility that this was due to the higher concentrations of FFA in plasma during hyperinsulinemia in the less sensitive subjects. During the time of the glucose uptake measurement (120–150 min), serum FFA averaged 189 and 140  $\mu\text{mol/l}$  ( $P < 0.02$ ) in the groups with normal and high insulin sensitivity, respectively. Could the 49  $\mu\text{mol/l}$  difference in FFA concentrations explain the 70  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  difference in glucose uptake in the exercising leg during hyperinsulinemia? This result is highly unlikely because we have previously shown acute lowering of FFA by 400  $\mu\text{mol/l}$  to increase skeletal muscle glucose uptake by 10  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  (45). We have also recently quantitated femoral muscle FFA uptake under similar hyperinsulinemic conditions as those prevailing in the present study (46). From these data, it can be calculated that the 49  $\mu\text{mol/l}$  difference in FFA concentrations corresponds to a difference of 0.042  $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$  in FFA uptake. Even if all FFAs were oxidized, this amount would consume only a trivial amount of oxygen (0.02 ml oxygen  $\cdot \text{kg}^{-1}$  muscle  $\cdot \text{min}^{-1}$ ) (47) compared with that in the resting or exercising muscles in the present study.

We measured counterregulatory hormone and lactate concentrations to ensure that the metabolic milieu during exercise- and insulin-stimulated glucose uptake measurements was similar in both groups. Regarding the insulin counterregulatory hormones, concentrations of those known to have effects on glucose uptake (i.e., cortisol, growth hormone, and catecholamines) were measured (Fig. 7). Serum cortisol concentrations were slightly higher at baseline in the group with high compared with normal insulin sensitivity, but neither these nor the concentrations during hyperinsulinemia or exercise periods differed significantly between the groups. Serum growth hormone concentrations increased slightly in both groups, but the magnitude of this increase was small compared with biologically significant changes such as those observed during hypoglycemia (48). Plasma adrenaline concentrations increased slightly during the exercise periods, but the increases were quantitatively trivial (49) with no significant differences between the groups. Blood lactate levels were also comparable between the groups both basally and during the hyperinsulinemic and exercise periods. In the entire group, blood lactate concentrations increased slightly but significantly. This increase could reflect either stimulation of glucose disposal by insulin, since blood lactate concentrations are directly proportional to the rate of glucose metabolism under euglycemic conditions (50), or the superimposed exercise.

In the present study, the groups were matched with respect to BMI and had negative family histories for diabetes; therefore, neither obesity nor genetic factors were likely to explain the difference in insulin sensitivity. The

group with high insulin sensitivity had higher  $\dot{V}O_{2\max}$ , which makes a difference in training status—the most likely explanation for the difference in insulin sensitivity. If neither FFA nor counterregulatory hormones or lactate concentrations explained the greater exercise-induced glucose extraction in the group with high compared with normal insulin sensitivity, the remaining possibility is that differences in intracellular signaling pathways between the groups were responsible. It is clear that acute contractions do not stimulate tyrosine phosphorylation of the insulin receptor or of IRS-1 (4). This finding also appears to apply to trained human muscle because Dela et al. (51) found both insulin binding and insulin-stimulated insulin receptor tyrosine kinase activity to be superimposable in trained and untrained muscle in subjects who had participated in single-leg bicycle training. The concentration of GLUT4 was however significantly higher in the trained leg than in the untrained leg. The exact mechanisms of how exercise translocates GLUT4 are unclear. It is of interest that although neither acute exercise nor training changes early insulin signaling mediators, the fractional activity of glycogen synthase increases more in response to in vivo insulin stimulation in trained subjects than in untrained subjects (6). Because glycogen synthase as well as GLUT4 are thought to be located downstream of the insulin- but not contraction-sensitive PI 3-kinase-dependent pathway (52), exercise- and insulin-stimulated glucose uptake do appear to share some common elements. Such similarities, which exact nature remain to be characterized, might have contributed to enhanced exercise-induced glucose uptake in the individuals with high insulin sensitivity. Of note, the present study was designed to establish in vivo whether enhanced glucose uptake in response to insulin is accompanied by an increase in the ability of exercise to stimulate glucose uptake under conditions in which the hormonal and metabolic milieu was maintained similar for both stimuli. These experimental conditions are not necessarily pertinent to those characterizing normal physiological conditions because exercise is not usually performed under hyperinsulinemic conditions, as performed in the present study and the studies of Dela et al. (13) and DeFronzo et al. (9).

In conclusion, when examined in a similar metabolic milieu in vivo, exercise increases glucose uptake more in individuals with high versus normal insulin sensitivity. These data suggest that although insulin and exercise both can independently stimulate glucose uptake, there are also similarities that enable insulin-sensitive individuals to increase their glucose uptake more in response to exercise than is possible in less insulin-sensitive individuals.

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