Mechanism of Enhanced Insulin Sensitivity in Athletes

Increased Blood Flow, Muscle Glucose Transport Protein (GLUT-4) Concentration, and Glycogen Synthase Activity

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Abstract

We examined the mechanisms of enhanced insulin sensitivity in 9 male healthy athletes (age, 25 ± 1 yr; maximal aerobic power [$\dot{V}O_2$max], 57.6 ± 1.0 ml/kg per min) as compared with 10 sedentary control subjects (age, 28 ± 2 yr; VO_2max, 44.1 ± 2.3 ml/kg per min). In the athletes, whole body glucose disposal (240-min insulin clamp) was 32% ($P < 0.01$) and nonoxidative glucose disposal (indirect calorimetry) was 62% higher ($P < 0.01$) than in the controls. Muscle glycogen content increased by 39% in the athletes ($P < 0.05$) but did not change in the controls during insulin clamp. VO_2max correlated with whole body ($r = 0.60$, $P < 0.01$) and nonoxidative glucose disposal ($r = 0.64$, $P < 0.001$). In the athletes forearm blood flow was 64% greater ($P < 0.05$) than in the controls, whereas their muscle capillary density was normal. Basal blood flow was related to VO_2max ($r = 0.63$, $P < 0.05$) and glucose disposal during insulin infusion ($r = 0.65$, $P < 0.05$). The forearm glucose uptake in the athletes was increased by 3.3-fold ($P < 0.01$) in the basal state and by 73% ($P < 0.05$) during insulin infusion. Muscle glucose transport protein (GLUT-4) concentration was 93% greater in the athletes than controls ($P < 0.01$) and it was related to VO_2max ($r = 0.61$, $P < 0.01$) and to whole body glucose disposal ($r = 0.60$, $P < 0.01$). Muscle glycogen synthase activity was 33% greater in the athletes than in the controls ($P < 0.05$), and the basal glycogen synthase fractional activity was closely related to blood flow ($r = 0.88$, $P < 0.001$). In conclusion: (a) athletes are characterized by enhanced muscle blood flow and glucose uptake. (b) The cellular mechanisms of glucose uptake are increased GLUT-4 protein content, glycogen synthase activity, and glucose storage as glycogen. (c) A close correlation between glycogen synthase fractional activity and blood flow suggests that they are causally related in promoting glucose disposal. (*J. Clin. Invest.* 1993. 92:1623–1631.) Key words: exercise • insulin sensitivity • glycogen synthase • blood flow • athletes

Introduction

Physical training enhances insulin-stimulated glucose disposal in proportion to the improvement in physical fitness (1, 2). A variety of factors may contribute to this increment in insulin sensitivity. First, physical training leads to changes in body composition with a reduction in fat and an increase in muscle tissue. The shift in body composition from adipose to muscular type enhances whole body glucose disposal (3). However, if calculated per muscle mass, the main target of insulin-stimulated glucose disposal (4), glucose uptake, is elevated only in aerobically trained athletes (e.g., runners), but not in subjects with anaerobic training (e.g., weight lifters) (3). Second, recent data in various patient groups suggest a novel mechanism for insulin resistance: a decreased effect of insulin to stimulate blood flow (5–8). There is a possibility that in the aerobically trained athletes the opposite mechanism enhances insulin sensitivity: blood flow is increased leading to augmented glucose disposal. In keeping with this hypothesis, in aerobically trained individuals muscle capillarization can be increased (9). When this is the case, it may lead to increased blood mean transit time and thus enhance tissue exposure to insulin and glucose. Insulin binding to muscle vascular endothelium and uptake of insulin into muscle tissue appears to be proportional to the degree of capillarization (10). Third, if a higher than normal amount of glucose is provided to the muscle tissue in the athletes by increased blood flow, muscle cells should have a mechanism capable of disposing the increased amount of glucose. This mechanism could be an increased amount of glucose transporter (11) and enhanced activity of metabolic enzymes. Studies in exercise-trained rats have indeed demonstrated an increase in the number of glucose transporters both in adipocytes (12) and in skeletal muscle (13–17). On the other hand, chronic physical inactivity (18) or limb denervation (19) decreases muscle glucose transporters in the rat. Data on the effects of physical training on glucose transporters in humans are scanty. Houmard et al. (20) reported increased amounts of muscle glucose transporters in trained middle-aged men. They did not study, however, whether there is any relationship between glucose transporters and factors such as glucose disposal and metabolism, blood flow, or physical fitness in these individuals.

Thus, a mechanism could exist consisting of increased blood flow, capillarization, and glucose transport proteins to regulate enhanced glucose disposal in the athletes. Since glucose demands may vary depending on the need to replace depleted glycogen stores or to provide energy via the oxidative pathway, the mechanism should be autoregulatory to orches-

trate the optimal disposal of glucose into the muscle tissue and its intracellular metabolism. The current study was planned to examine various components of this possible network contributing to enhanced insulin sensitivity in the athletes. In particular, we studied blood flow, capillary density, glucose transport proteins, glucose disposal and its oxidative and nonoxidative metabolism, and their interrelationship in healthy subjects with a wide range of physical fitness.

Methods

Subjects. Nine well-trained male athletes (bandy players: age, 25.4±1.2 yr; body mass index [BMI], 23.2±0.6 kg/m²) and 10 untrained control subjects (age, 28.0±2.1 yr; BMI, 23.9±0.9 kg/m²) participated in the study. All the subjects were healthy and none of them were using any medication. For 2 d before the study, the subjects did not participate in any training and ingested 250–300 g/d of carbohydrates. The purpose, nature, and possible risks of the study were explained to all subjects before informed consent was obtained. The study protocol was approved by the Ethical Committee of the Helsinki University Hospital.

Design. Design of the study is shown in Fig. 1. In each subject, we performed a 240-min euglycemic insulin clamp. Indirect calorimetry, forearm blood flow, and glucose disposal were determined throughout the study, and muscle biopsies were performed before and at the end of the clamp. The following measurements were done: whole body total, oxidative and nonoxidative glucose disposal, muscle blood flow, and glucose disposal in forearm. In biopsy specimens from m. quadriceps vastus lateralis, we determined glycogen and glycogen synthase and its mRNA and capillary density. From the baseline samples only, we measured glucose transport protein (GLUT-4) and its mRNA concentration. In addition, maximal aerobic power was determined in each subject 1–2 wk before the clamp study.

Maximal aerobic power ($\dot{V}O_2max$). This was determined using a work-conducted upright exercise test with an electrically braked cycle ergometer (model ERG 220; Robert Bosch, GmbH, Berlin, Germany) combined with continuous analysis of expiratory gases and minute ventilation (EOS-Sprint; Erich Jaeger GmbH, Wuertzburg, Germany). The exercise was started after a 10-min rest with a work load of 50 W. It was then increased stepwise by 50 W every 3 min until exhaustion (grade 19–20/20 according to the Borg scale of perceived exertion) (21). For collection of respiratory gases a tightly attached face mask (Rudolpf series 7910; Hans Rudolph Inc., Wyandott, Kansas City, MO) with a dead space of 185 ml was used. The mixed expiratory gas was sampled for analysis and O_2 and other gas exchange parameters (CO_2, respiratory quontient) were automatically calculated and

INSULIN AND GLUCOSE INFUSION

ICM   ICM   ICM   ICM

FLOW AND FOREARM GLUCOSE DISPOSAL

-40   0   60   120   180   240 min
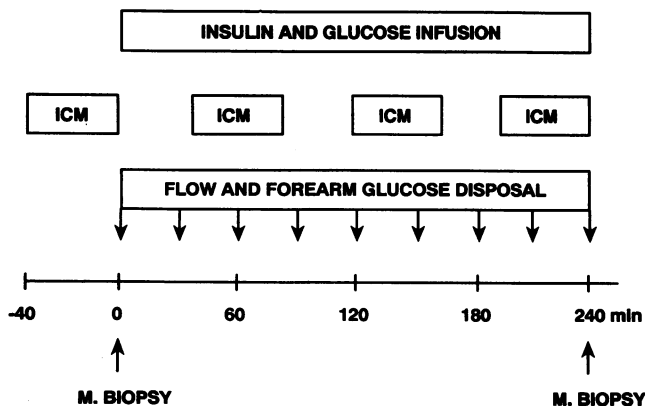
M. BIOPSY   M. BIOPSY

Figure 1. Design of the study. ICM, indirect calorimetry. Insulin was infused at a rate of 9 nmol or 1.5 mU/kg per min. Variable rate of 20% glucose infusion was used to maintain euglycemia.

printed out at 30-s intervals. Maximal aerobic power was defined as $\dot{V}O_2$ of the highest 30 s of the exercise. Maximal exercise levels obtained exceeded the anaerobic threshold as indicated by the fact that the respiratory gas exchange ratio ($\dot{V}CO_2/\dot{V}O_2$) was > 1.10 at the end of exercise in all subjects.

Whole body insulin-stimulated glucose disposal or insulin sensitivity. This was determined using the euglycemic insulin clamp technique, as previously described (1, 22). After an overnight fast, three catheters were inserted: one for an antecubital vein for the infusion of insulin and glucose, one in an ipsilateral antecubital deep vein for the blood sampling and blood flow determinations, and the third into a heated (60°C) hand vein for the sampling of arterialized blood. Insulin (actrapid human; Novo Nordisk, Copenhagen, Denmark) was infused in a primed continuous rate (9 nmol [1.5 mU]/kg/min) for 240 min. Plasma glucose was maintained at the fasting level with 20% glucose infused at variable rate based on plasma glucose determinations from the arterialized blood at 5–10-min intervals. Since hepatic glucose production is totally suppressed with even lower insulin infusion rates than used here (23), the glucose infusion rate corrected for changes in plasma glucose concentration equals the rate of glucose disposal. The rate of glucose disposal is expressed per lean body mass (LBM). LBM was calculated from the equation: LBM = {6.493 + [0.4936 × (height²/resistance)] + (0.332 × weight)(kg)}. The total body resistance was determined with a four-terminal portable impedance analyzer as previously described (24).

Oxidative glucose disposal. This was determined with indirect calorimetry (the Deltatrac Metabolic Monitor; Datex, Helsinki, Finland) in the basal state and three times during the insulin clamp study (Fig. 1), as previously described from our laboratory (25, 26). The constants to calculate glucose and lipid oxidation from respiratory exchange data have been given previously (27). Nonoxidative glucose disposal (storage) is defined as the difference between total and oxidative glucose disposal.

Forearm blood flow. This was determined with capacitance plethysmography (model 2560; UFI, Morro Bay, CA) as previously described (25, 26). Briefly, hand blood flow was first excluded by high (250 mmHg) pressure. Thereafter forearm venous flow was excluded, and at the start of a rise in voltage on the recorder chart, blood was drawn into the syringe through a catheter inserted retrogradely into a deep vein. Blood was drawn at a rate that prevented any further change in voltage. Thus, the rate blood is drawn into the syringe equals the rate of arterial blood flow into the arm. After ∼ 10 ml of blood was drawn, the determination was discontinued and both the arm flow and forearm venous flow were reopened. Forearm volume was determined by water displacement. Due to problems with small veins in five subjects, flow was determined in seven athletes and seven control subjects.

Forearm glucose disposal. Disposal (μmol/ml forearm/min) was determined (Fig. 1) by multiplying total forearm blood flow (ml/ml forearm per min) by the arterialized venous blood − deep venous blood difference (mmol/liter) for glucose as determined from a single pair of blood samples taken at 30-min intervals as previously described (25, 26). Hand blood flow was excluded before the blood sample from deep vein was obtained. The proper location of the catheter in deep vein was confirmed by the increase in the glucose A-V difference from the basal value of 0.2±0.01 mM (range, 0–0.5 mM) to the maximum value of 2.2±0.04 mM (range, 1.6–3.0 mM) during insulin infusion.

Muscle biopsies. 15 min before and at the end of 240-min insulin clamp study, a percutaneous muscle biopsy (100–150 mg) was performed with a Bergström needle under local anesthesia (1% lidocaine). The samples were obtained from m. vastus lateralis from opposite sites before and after the study. The specimen was removed from muscle with suction by a syringe attached to the needle, and the needle was immediately emptied into liquid N_2. With this procedure, the muscle sample can be frozen in < 5 s after removal. The specimens were stored in liquid N_2 until analyzed.

Capillary density. Under a microscope at −20°C, the frozen samples were mounted in an embedding medium (Tissue-Tek O.C.T. compound; Miles Inc., Elkhart, IN). Transverse sections (10 μm) were cut with a microtome at −20°C and stained with the amylase-periodic

acid Schiff method to visualize capillaries (28). The sections were photographed and copied (magnification, 200). On the photographs, one or several areas without artifacts produced by the sectioning and staining procedure and without tendencies towards longitudinal cuts or connective tissue structures splitting up the section in different parts were framed by following the cell borders. Capillaries per fiber (the number of capillaries divided by the number of muscle fibers), capillaries per square millimeter, and the mean fiber area were determined as described in detail previously (29). In each biopsy an average cross-sectional area of 0.85 ± 0.10 mm^2 (mean$\pm$SEM) was studied (containing 128 ± 14 muscle fibers). Nine of the subjects were studied with duplicate biopsies (mean of pre- and post-clamp), whereas in the remaining seven only one of the biopsies could be used. In three subjects, none of the muscle samples could be processed to permit reliable estimation of capillarity.

Preparation of tissue RNA, and quantitation of muscle DNA, GLUT-4 mRNA, glycogen synthase mRNA, and GLUT-4 protein levels. Total tissue RNA was extracted with the guanidine thiocyanate water-saturated phenol extraction method (30). Samples were homogenized in 4 M quanidine thiocyanate containing 1% of octophenol-ethyleneoxide detergent (NP-40; Sigma Chemical Co., St. Louis, MO, USA), and multiple water-saturatedphenol/phenol-chloroform-isoamylalcohol/chloroformextractions and ethanol precipitation were performed. After determining the absorbance at 260 and 280 nM, aliquot of RNA was run on minigel to verify the integrity of RNA preparations. The $A_{260/280}$ ratios were ~ 2 for all samples. Aliquots of total RNA (5.0 and 10.0 μg) were dissolved in 5.6% formaldehyde, 10$\times$ SSC (1.5 mol/liter NaCl, 0.015 mol/liter Na-citrate, pH 7.0) and blotted onto Hybond-N membranes (Amersham International, Amersham, UK). Membranes were hybridized with the appropriate probes ($\sim 5 \times 10^6$ cpm/ml) for 18 h at 60°C in 50% formamide, 5$\times$ SSPE (0.75 mol/liter NaCl, 50 mmol/liter NaH$_2$PO$_4$, 5 mmol/liter EDTA), 3$\times$ Denhardt's 0.1% SDS, and then washed in 0.1$\times$ SSC, 0.1% SDS at 65°C. The blots were exposed to Fuji RX film (Fuji Photo Film, Tokyo, Japan) at $-70°$ using intensifying screens (Cronex Lightning Plus; DuPont Co., Wilmington, DE) for various periods of time. The amount of GLUT-4 and glycogen synthase mRNA present in each sample was measured by densitometric analysis, comparing the intensity of sample dots with dilutions of a known amount of appropriate cDNA standard dots as described (31). A 1.7-kb fragment of GLUT-4 cDNA from a human jejunal library was subcloned into Bluescript SK$^+$ plasmid (Stratagene, La Jolla, CA) as described (31) and used as template for transcription of ^{32}P-labeled cRNA probe with T7 RNA polymerase according to the manufacturer's recommendations. Dilutions of known amounts of GLUT-4 cDNA were used as standards. The template for glycogen synthase cRNA probe was a HINDIII-linearized Bluescript SK$^+$ plasmid containing human glycogen synthase cDNA and the cDNA insert was used as standard (32). DNA concentrations of tissue homogenate were measured with a DNA fluorometer (model TKO 100; Hoefer Scientific, San Francisco, VA) with Hoechst dye (model 33258; Poly-Sciences, Warrington, PA) and calf thymus DNA (Sigma Chemical Co.) as standard.

Immunoblotting. Muscle was homogenized (Polytron, Brinkman, Westbury, NY) on ice at high speed for 45 s in HES buffer (20 mM Hepes, 1 mM EDTA, 250 mM sucrose, pH 7.4, 1:40; wt/vol). The protein concentration was determined by a method based on the reaction with bicinchoninic acid (Pierce, Rockford, IL), and 50-μg samples were subjected to SDS-PAGE. Protein was electrophoretically transferred to nitrocellulose paper (MilliBlot-SDE System; Millipore, American Bionetics, Bedford, MA). Nitrocellulose sheets were incubated with phosphate-buffered saline (pH 7.4) containing 50 mg/ml of powdered milk (Valio Ltd., Helsinki, Finland) for 60 min at 22°C, and thereafter with a polyclonal antibody specific for the GLUT-4 carboxy terminus (F349), and after washing with ^{125}I-labeled donkey anti–rabbit IgG antibody (Amersham Corp., Arlington Heights, IL). Quantitation was performed by generation of autoradiographs and performance of densitometry against a gray scale standard using standard software (Image 1.32f; National Technical Information Service, Springfield, VA).

Muscle glycogen and glycogen synthase assays. These were done as previously described (25, 26, 33). The samples were freeze-dried and dissected free from connective tissue and blood. The muscle was powdered and divided into two lots, which were weighed and used for glycogen and metabolite determination. 3–5 mg of muscle powder was extracted with KOH, neutralized, glycogen was hydrolyzed with amyloglycosidase, and the liberated glucose was analyzed by the glucose oxidase method. Glycogen concentration is expressed as millimoles per kilogram of dry muscle (25). Glycogen synthase activity was measured by a fluorometric method as described previously (34, 35). This method determines the production of uridine diphosphate (UDP) from the incorporation of UDP-glucose in glycogen. The final concentration of UDP-glucose added to the solution was 7.1 mM. Samples and blanks were incubated for 15 min at 37°C with 0.1 and 10 mM glucose-6-phosphate (G-6-P). The assay was linear for protein concentration in the homogenate. Protein concentration was determined according to Lowry et al. (36). The enzyme activity as determined at 0.1 mM G-6-P is called glycogen synthase activity, the one at 10 mM G-6-P is called total glycogen synthase activity, and the ratio of the two (0.1 mM/10 mM G-6-P) is called glycogen synthase fractional activity. Due to the small muscle sample size in four subjects, glycogen synthase activity was determined in eight athletes and seven control subjects.

Other determinations. Glucose in the plasma and glycogen assays was determined with a glucose oxidase method using a glucose analyzer (Beckman Instruments, Fullerton, CA). The determinations of free fatty acid (FFA) concentrations in plasma were done by using the fluorometric method of Miles et al. (37). Serum insulin was measured by a double antibody radioimmunoassay (Pharmacia, Uppsala, Sweden) (38).

Statistical methods. Comparison between the groups was done with Mann-Whitney's two-sample rank sum test. Wilcoxon-Pratt's test was used in the calculations between paired items. The correlation analysis was done with Spearman's test and the trends with Friedman's test. Kendall's partial rank correlation coefficient was used to calculate the effect of a third variable on the interrelationship of two factors. P values < 0.05 were considered significant. The results are given as mean$\pm$SE.

Results

Maximal aerobic power and body composition. In the athletes, $\dot{V}O_2$max was higher than in the controls (57.6 ± 1.0; range, 53.4–61.6 vs. 44.1 ± 2.3; range, 29.0–52.7 ml/kg per min, respectively, $P < 0.001$). Lean body mass was not significantly different between the athletes (65.9 ± 1.3 kg) and the control subjects (66.4 ± 1.6 kg). Body fat percent was slightly, although not significantly, lower in the athletes ($11.6\pm0.9\%$) than in the control subjects ($13.6\pm1.2\%$).

Glucose disposal. In the basal state, plasma glucose (5.7 ± 0.1 vs. 5.8 ± 0.1 mM), serum insulin (16.3 ± 2.1 vs. 19.2 ± 2.1 pM), and glucose oxidation rate (7.8 ± 1.0 vs. 8.2 ± 0.8 μmol/kg per min) were all similar in the athletes and control subjects, respectively. During hyperinsulinemia, steady-state plasma glucose (5.2 ± 0.1 vs. 5.2 ± 0.1 mM) and insulin concentrations (669.7 ± 19.0 vs. 681.5 ± 29.1 pM) were similar in the athletes and the control subjects, respectively. The total body glucose disposal per lean body mass in the athletes (75.8 ± 2.9 μmol/kg per min) was 32% higher than in the control subjects (57.3 ± 4.8 μmol/kg per min, $P < 0.01$; Fig. 2). The entire difference in systemic glucose disposal between the groups was due to a 62% higher nonoxidative glucose disposal in the athletes (50.8 ± 2.4 μmol/kg per min) as compared with the controls (31.4 ± 3.6 μmol/kg per min; $P < 0.001$), whereas glucose oxidation rate was similar in the two groups.

When both groups were combined, total body glucose disposal rate correlated with $\dot{V}O_2$max ($r = 0.60$, $P < 0.01$; Fig. 3
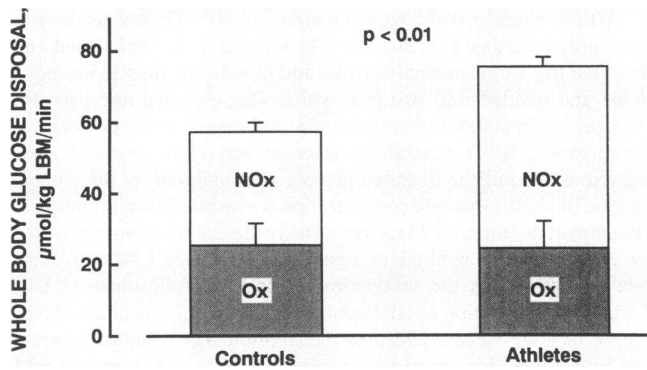
Figure 2. The rate of whole body glucose uptake (*entire bar*) and nonoxidative (*NOx*) and oxidative (*Ox*) glucose metabolism in the athletes and sedentary control subjects.

A). This correlation is accounted for by the relationship between nonoxidative glucose disposal and $\dot{V}O_2$max ($r = 0.64$, $P < 0.001$; Fig. 3 *B*), since no correlation was observed between glucose oxidation and maximal aerobic power ($r = 0.07$).

Forearm blood flow and glucose disposal. In the basal state, the forearm blood flow in the athletes (0.0326 ± 0.0040 ml/ml forearm per min) was 64% greater than in the control subjects (0.0199 ± 0.0026 ml/ml forearm per min; $P < 0.05$; Fig. 4). The blood flow in the basal state correlated both with $\dot{V}O_2$max ($r = 0.63$, $P < 0.05$) and with the glucose disposal rate ($r = 0.53$, $P < 0.05$). Insulin infusion did not significantly change the flow in the control group (change 0.010 ± 0.004 ml/ml forearm per min; $P = 0.06$, vs. basal) or in the athletes
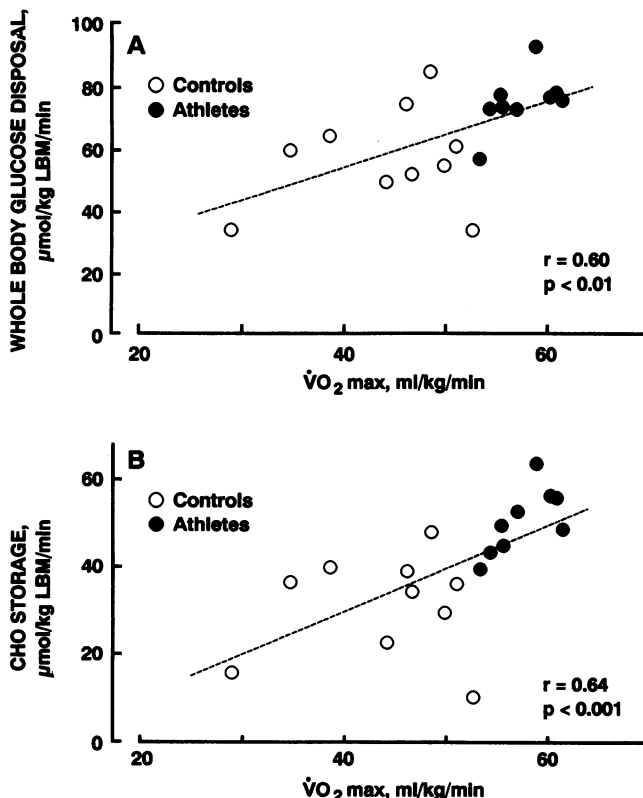

Figure 3. Correlation between maximal aerobic power ($\dot{V}O_2$max) and whole body glucose disposal (*A*) or glucose storage (*B*) in the whole group studied.
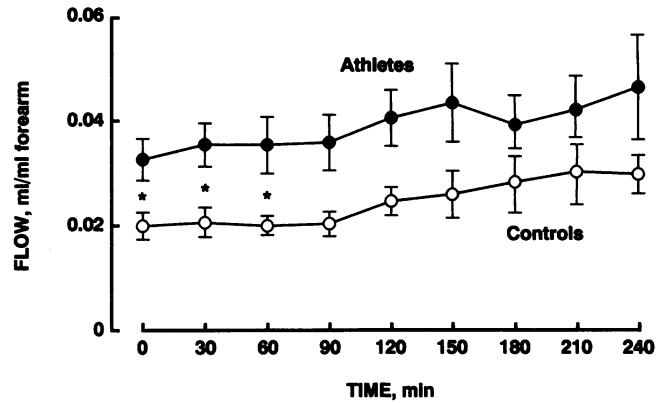

Figure 4. Forearm blood flow in the basal state and during insulin infusion in the athletes and sedentary control subjects. The difference is significant at time points as indicated (*$P < 0.05$) and also for the mean values between the groups ($P < 0.05$).

(0.014 ± 0.009 ml/ml forearm per min, $P = 0.22$ vs. basal). The mean flow throughout the study (0–240 min) was in the athletes greater than in the control subjects ($P < 0.05$). There was a close correlation between forearm blood flow and total body glucose disposal rate in the whole group ($r = 0.86$, $P < 0.001$; Fig. 5).

In the basal state, the plasma glucose A-V difference in the athletes (325 ± 47 μmol/liter) was 2.2-fold greater than in the untrained subjects (151 ± 30 μmol/liter; $P < 0.05$; Fig. 6 *A*). Consequently, the average basal glucose disposal rate (flow $\times$ A-V difference) in the basal state was 3.3-fold greater in the athletes than in the sedentary subjects (10 ± 1 vs. 3 ± 1 μmol/kg per min; $P < 0.01$; Fig. 6 *B*). The basal forearm glucose disposal correlated both with $\dot{V}O_2$max ($r = 0.64$, $P < 0.05$) and with insulin-stimulated total body glucose uptake ($r = 0.65$, $P < 0.05$). The basal A-V difference correlated also with the glucose disposal during insulin infusion ($r = 0.63$, $P < 0.05$).

During insulin infusion (from 60 to 240 min), the imparity of glucose A-V differences disappeared and the curves were nearly superimposable in the two groups (Fig. 6 *A*). However, the A-V difference started to decrease from 60 min, and the trend to 240 min was significant in the whole group ($P < 0.01$) and in the control subjects ($P < 0.05$), but not in the athletes. Due to a greater flow, the average insulin-stimulated forearm glucose disposal in the athletes (64 ± 6 μmol/kg per min) was 73% greater than in the sedentary subjects (37 ± 5 μmol/kg per
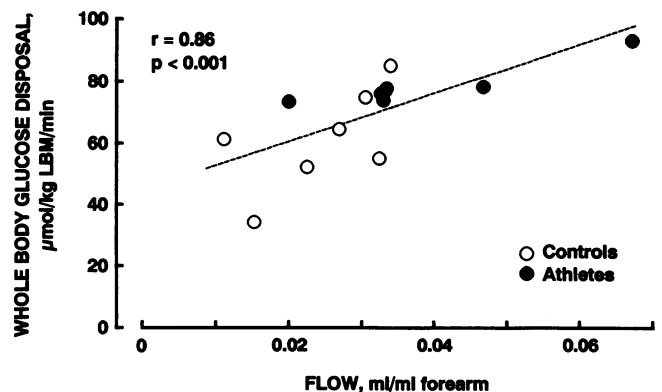

Figure 5. Correlation between whole body glucose disposal and forearm blood flow.
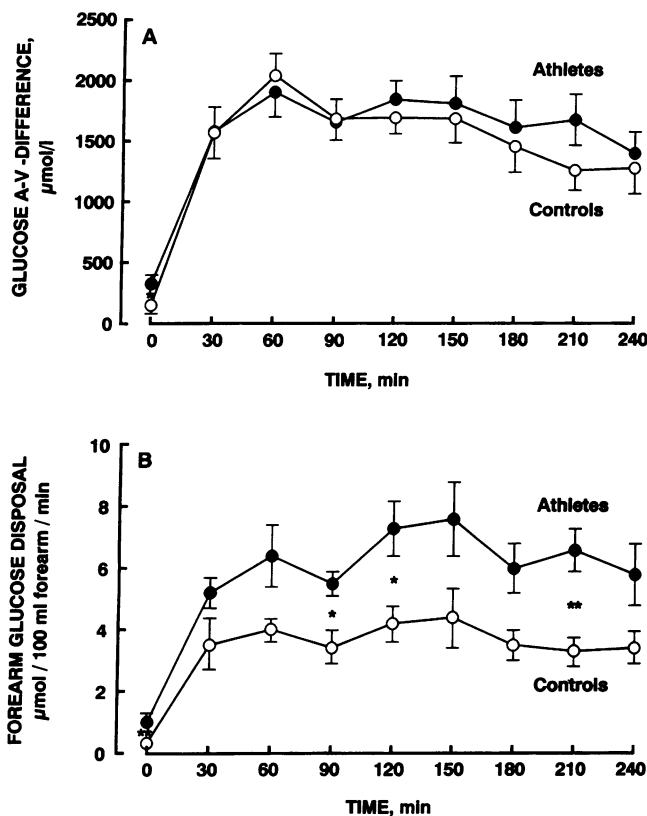
Figure 6. The difference in the arterialized vein–deep vein plasma glucose concentration (A) and forearm glucose uptake (B) in the athletes and sedentary control subjects. $*P < 0.05$; $**P < 0.01$ between athletes and controls.

min, $P < 0.05$). When calculated at 30-min periods from 60 to 240 min of insulin infusion there was an inverse correlation between forearm blood flow and glucose A-V difference both in the sedentary subjects ($r = -0.46$, $P < 0.001$) and in the athletes ($r = -0.52$, $P < 0.001$).

Muscle glycogen and glycogen synthase

Glycogen. In the basal state, muscle glycogen concentrations were not significantly different in the athletes and sedentary control subjects (282 ± 36 vs. 351 ± 27 mmol/kg dry muscle). During the 4-h insulin infusion, glycogen content increased in the athletes by 39% (to 393 ± 23 mmol/kg dry muscle; $P < 0.05$), but did not change significantly in the sedentary sub-

jects (383 ± 29 mmol/kg dry muscle). There was a close inverse relationship between basal glycogen content and the increase in glycogen during the clamp ($r = -0.73$, $P < 0.001$).

Glycogen synthase. In the basal state the glycogen synthase activity was higher in the athletes than in the controls (Table I). There was also a close relationship between glycogen synthase activity and total glycogen synthase activity both in the basal state ($r = 0.68$, $P < 0.01$) and after insulin stimulus (Fig. 7). Both the basal glycogen synthase activity ($r = -0.64$, $P < 0.01$) and glycogen synthase fractional activity ($r = -0.92$, $P < 0.0001$) were inversely related to basal muscle glycogen content. Also, basal glycogen synthase fractional activity correlated with glucose storage ($r = 0.63$, $P < 0.05$) and the rise in muscle glycogen content ($r = 0.75$, $P < 0.01$), but not with total glucose disposal during the clamp. The mean forearm blood flow (0–240 min) correlated with the glycogen synthase fractional activity as determined in the basal state ($r = 0.88$, $P < 0.001$) or after insulin stimulus ($r = 0.82$, $P < 0.01$). However, when basal glycogen synthase fractional activity was taken as a constant, Kendall's partial rank correlation revealed there was no direct association between the flow and postclamp glycogen synthase fractional activity ($P = 0.46$). Thus, the relationship was only between the basal glycogen synthase fractional activity and the flow. In addition, basal glycogen synthase activity was directly related to the increase in blood flow during the insulin infusion ($r = 0.59$, $P < 0.05$).

Glycogen synthase mRNA. The glycogen synthase mRNA was similar in the athletes and control subjects both in the basal state (0.37 ± 0.15 vs. 0.59 ± 0.24 pg/μg RNA) and after insulin infusion (0.71 ± 0.43 vs. 0.44 ± 0.14 pg/μg RNA). The glycogen synthase mRNA concentration did not change significantly in either group.

GLUT-4 protein. Muscle GLUT-4 protein concentration, as expressed per muscle protein, was 93% greater ($P < 0.01$) in the athletes than sedentary control subjects (Fig. 8). This difference was not due to variations in the muscle protein content, since that was similar in the athletes and controls (0.10 ± 0.03 vs. 0.12 ± 0.03 mg/mg muscle, respectively). The GLUT-4 protein concentration correlated with $\dot{V}O_2$max ($r = 0.61$, $P < 0.01$; Fig. 9). Moreover, there was a significant association between the total body glucose disposal and GLUT-4 concentration ($r = 0.60$, $P < 0.01$; Fig. 10). GLUT-4 protein concentration was related to the rate of nonoxidative glucose disposal ($r = 0.66$, $P < 0.01$), but not to the glucose oxidation ($r = 0.10$, NS). According to Kendall's partial rank correlation coefficient, the association of GLUT-4 protein and total body glucose disposal was independent of blood flow.

Table I. Glycogen synthase activity and Its mRNA as Determined in m. Quadriceps Femoris in the Athletes and Untrained Control Subjects in the Basal State and after 240 min Hyperinsulinemia

	Basal		Hyperinsulinemia	
	Controls	Athletes	Controls	Athletes
Glycogen synthase activity (nmol/min per mg prot.)				
Glucose-6-phosphate				
0.1 mM	24.3 ± 2.4	$36.9\pm5.4*$	32.2 ± 5.4	40.2 ± 4.8
10.0 mM	44.5 ± 4.3	53.4 ± 6.8	45.6 ± 6.7	52.0 ± 5.1
Fractional activity	0.55 ± 0.03	0.69 ± 0.05	$0.70\pm0.02^{\ddagger}$	0.77 ± 0.02
Glycogen synthase mRNA (pg/μg RNA)	0.59 ± 0.24	0.37 ± 0.15	0.44 ± 0.14	0.71 ± 0.43

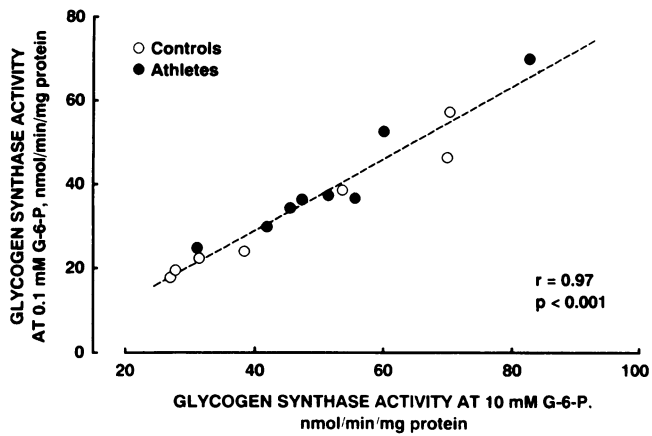$* P < 0.05$ vs. controls. $^{\ddagger} P < 0.05$ vs. basal.

Figure 7. Correlation between glycogen synthase activity and glycogen synthase total activity after insulin clamp.

GLUT-4 mRNA. GLUT-4 mRNA concentration was similar in the athletes and the control subjects (Fig. 8). There was no correlation between GLUT-4 mRNA and protein content or glucose disposal during the insulin clamp.

Free fatty acids. There were no differences in the baseline FFA concentrations between the athletes and sedentary subjects, respectively (481 ± 59 vs. 427 ± 45 μmol/liter). The values decreased during the first 30 min of insulin infusion and continued to decline more slowly thereafter with no difference between the groups at the end of the clamp (95 ± 6 vs. 120 ± 9 μmol/liter).

Capillary density. Capillary density in m. quadriceps femoris was not different in the athletes (261 ± 17 capillaries/mm^2) and in the control subjects (293 ± 18 capillaries/mm^2). The mean muscle fiber area in the athletes ($7,898\pm757$ μm^2) was 48% greater than in the control subjects ($5,343\pm827$ μm^2), although this difference was not significant. However, the fiber area correlated positively with the glucose disposal rate ($r = 0.67$, $P < 0.01$) and with the $\dot{V}O_2$max ($r = 0.55$, $P < 0.05$), and negatively with the capillary density ($r = -0.77$, $P < 0.01$). The capillary/fiber ratio was related to $\dot{V}O_2$max ($r = 0.51$, $P < 0.005$). There was no correlation between capillary density and blood flow or whole body glucose disposal.

Additional control group. Since in the athletes of the present study muscle glycogen content was slightly (20%), although not significantly, lower than in the sedentary subjects,
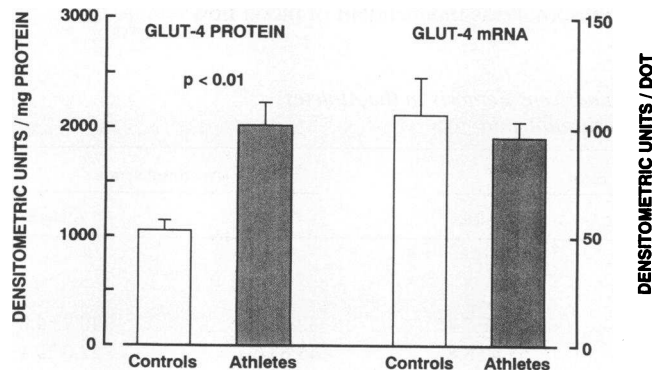

Figure 8. Concentration of muscle glucose transport protein (GLUT-4), densitometric U/mg protein) and its mRNA (densitometric U/dot) concentration in m. quadriceps femoris lateralis in sedentary control subjects and trained athletes in the basal state.
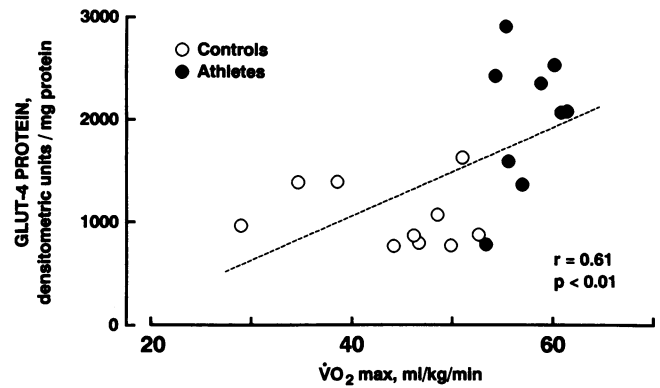
Figure 9. Correlation between maximal aerobic power ($\dot{V}O_2$max) and muscle GLUT-4 protein concentration.

we studied an additional control group to find out whether a moderate glycogen depletion caused by exercise on a previous day has an influence on body sensitivity to insulin. Six males (age, 33 ± 2 yr; BMI, 22.8 ± 0.3 kg/m^2; $\dot{V}O_2$max, 45.3 ± 2.0 ml/kg per min) participated in this study. They were examined twice: after a day when they exercised by running, and 2–3 wk later after a resting control day. On both days, the diet was similar with carbohydrate 250–300 g/d. In both studies, a 4-h insulin clamp and a muscle biopsy before the clamp were performed similarly as for the athletic and sedentary study groups. In these subjects, muscle glycogen content was 21% lower on the day after exercise as compared with the study after a resting, control day (234 ± 27 vs. 295 ± 53 mmol/kg dry muscle). This difference was not significant. Neither was there any significant difference in the whole body glucose disposal (57.3 ± 2.3 vs. 62.8 ± 2.7 μmol/kg per min) between the studies done after the exercise or after a resting control day, respectively.

Discussion

In the current study, the athletes were characterized by increased forearm blood flow, enhanced muscle GLUT-4 protein concentration, increased glycogen synthase activity, glycogen synthesis, and glucose storage as compared with the sedentary controls. Blood flow, GLUT-4 protein concentration, and nonoxidative glucose disposal were related to $\dot{V}O_2$max, suggesting that they are consequences of physical training. These changes can also explain, at least in part, the mechanisms of
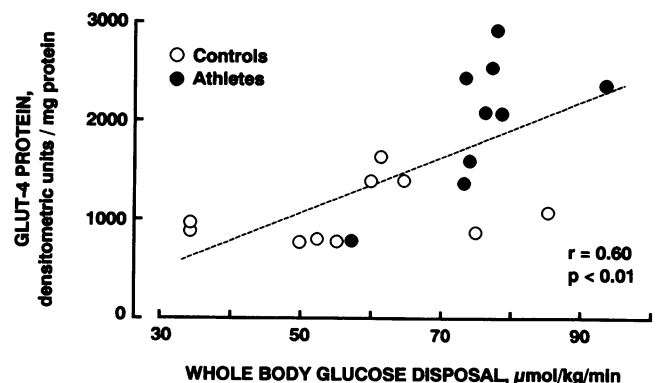

Figure 10. Correlation between whole body glucose disposal and muscle GLUT-4 protein.

augmented glucose disposal observed after physical training (1, 2, 33, 39, 40).

In cross-sectional studies, it is debated whether the increased insulin action in the athletes reflects a long-term adaptation or is a consequence of the latest acute bout of exercise (41, 42). A direct stimulatory effect of acute exercise on glucose disposal vanishes within a few hours (43), and the effect on insulin sensitivity disappears within 1–2 d (40, 44). The rate of reversal varies and depends, at least in part, on the refilling of glycogen stores (45). In the current study, the subjects were studied ≥ 2 d after the last exercise bout, whereafter they had ingested a high carbohydrate diet. At the time of the study, there was a slight (20%), but not significant, difference in muscle glycogen content between the athletic and sedentary control group. In the additional control group studied, a 21% (nonsignificant) glycogen depletion, which was induced by an exercise on the previous day, had no effect on insulin sensitivity. These data indicate that a difference in muscle glycogen content of this magnitude does not significantly alter glucose disposal in the athletes. Thus, even if the athletes had been physically more active than the controls, it is unlikely that differences in the physical activity shortly before the study, or a small difference in muscle glycogen content between the athletic and sedentary subjects, had any major contribution to the differences in glucose disposal between the two groups. More probably the increased insulin sensitivity represents a true long-term adaptation to training rather than a consequence of an acute exercise bout. This is supported by correlation of the nonoxidative glucose disposal rate to $\dot{V}O_2$max or to GLUT-4 protein, both of which reflect long-term adaptation to training. However, in the lack of elevated muscle glycogen stores, as described in the athletes after intensive training and dietary manipulation (46), we cannot totally exclude the influence of "a relative glycogen deficiency" on augmented glucose disposal in the athletes.

Blood flow was determined with plethysmography. With this technique, a total flow in the arm is determined. However, muscle blood flow represents a majority (80%) of total forearm flow (47). Thus, blood flow as determined with plethysmography should well represent muscle flow.

Regarding the enhanced forearm blood flow in our athletes, it existed already in the basal state, whereas the flow failed to increase significantly in either group during insulin infusion. Thus, while decreased blood flow response to insulin may contribute to insulin resistance in various pathologic conditions (5–8), in these athletes it is augmented blood flow in the basal state rather than in response to insulin that may contribute to their enhanced glucose delivery and disposal. The mechanism of enhanced blood flow in these athletes at rest is unclear. We are not aware of any data indicating increased resting cardiac output in the athletes. Previous studies with runners (48) or swimmers (49) have demonstrated a blood flow higher than normal during exercise, but normal in the resting state. In terms of the mechanisms of increased blood flow in our athletes, enhanced capillary growth has been described in trained muscles (29, 45, 50). However, training for speed does not necessarily lead to increased capillarization (51). Our athletes were bandy players. Bandy (a sport with similarities to ice hockey) is characterized by a high demand of aerobic processes, where the different actions require 70–100% (82% on the average) of the $\dot{V}O_2$max (52). In addition, the occasional high skating speed requires great leg strength, and bandy players have a 36% higher leg extension strength as compared with untrained controls (51). This difference is of a similar magnitude to the 48% increase in fiber area of the bandy players in the current study. The fiber area was related both to maximal aerobic power and glucose disposal rate, suggesting that training may have increased glucose disposal rate via an enlargement of fiber area. There is no corresponding information on other muscle groups. However, bandy requires strength also in the forearm muscles, which would be expected to be hypertrophied similarly to the quadriceps muscle. The relative degree of forearm muscle capillarization in the athletes can only be speculated, but the aerobic processes and consequent capillarization in the arm are probably less than in the leg. Thus, enhanced flow in the athletes cannot be explained by an anatomic enlargement of capillaries, but is more likely an increase in arteriolar function.

There was a close association between the mean forearm blood flow and the preclamp muscle glycogen synthase fractional activity as determined in the m. quadriceps lateralis in the leg. Moreover, the rise in blood flow was related to the glycogen synthase activity in the basal state. Whether there is a causal relationship between the basal muscle glycogen synthase fractional activity and blood flow during insulin infusion (either directly or mediated by a third factor, such as insulin) remains an interesting hypothesis for a positive feedback mechanism between glucose delivery and disposal in the muscle tissue. Blood flow and glucose disposal were determined across the forearm, whereas in vitro studies on muscle were performed on the leg. Although physical training in bandy involves both legs and arms, the exercise is more intense for leg muscles. Thus, it is possible that a correlation between glycogen synthase fractional activity and the flow would have been even stronger had the flow been measured in the leg.

Forearm glucose disposal in the athletes was 3.3-fold greater in the basal state and 73% greater during insulin infusion as compared with the sedentary controls. In the basal state, both greater flow and A-V difference contributed to the increased glucose disposal. During insulin infusion, the A-V difference was identical in the two groups, with an initial rise and a peak at 60 min. Thus, in the face of identical A-V difference, the greater glucose disposal in the athletes is accounted for by their greater blood flow. Since on average 60% of the forearm is muscle tissue (52) and muscle is the main target of insulin-stimulated glucose disposal (4), a majority of this difference is due to increased glucose disposal by muscle in the athletes. There was a close inverse relationship between the A-V difference and blood flow as determined at 30-min intervals after the first 60 min of insulin infusion. These data suggest a regulationship between glucose delivery (flow) and uptake (A-V difference) in order to provide a constant glucose flux into the muscle tissue. If insulin contributes to this regulation, it can do it by two different mechanisms: first, insulin could enhance glucose availability via arterial relaxation and increased blood flow (mediated by stimulation of glycogen synthase); and second, insulin could enhance glucose uptake and A-V difference. In the latter case, there is a compensatory decrease in blood flow to maintain constant glucose flux.

Regarding the cellular mechanisms of increased glucose uptake in the trained individuals, free fatty acid concentrations both in the basal state and during insulin infusion were similar in both groups. Thus, a difference in glucose fatty acid cycle may not explain different glucose uptake in the two groups (53, 54). Muscle GLUT-4 protein concentration was elevated by 93% in the athletes. This is in keeping with previous reports in trained animals (10–13) and in man (20). Moreover, the mag-

nitude of the increment in GLUT-4 protein was consistent with a 73% greater forearm glucose disposal in the trained than in untrained individuals. In addition, GLUT-4 concentration correlated with $\dot{V}O_2$ max, whole body glucose disposal, and glucose storage rate. These associations suggest that increased GLUT-4 concentration was a training effect and may contribute to enhanced glucose disposal. In keeping with the importance of GLUT-4 for mediating increased glucose disposal, recent data in the rat have demonstrated that training-induced changes are specific for GLUT-4 and not GLUT-1, and that the increase in the GLUT-4 protein occurs in the plasma membrane rather than in the intracellular pool (16). In addition, the training-induced rise in muscle glucose uptake is due to an increase in the number rather than in the intrinsic activity of glucose transporters (17). Moreover, in endurance-trained individuals the proportion of type I (red oxidative) is increased, and there is a positive correlation between the type I fiber content and GLUT-4 protein concentration (20). Physical training converts type IIb (white glycolytic) fibers into type IIa (red glycolytic) fibers (55). An inverse relationship has been reported between GLUT-4 protein content and the proportion of type IIb fibers in the muscle (20). In the current study, we did not determine muscle fiber content. It is thus possible that a shift from type IIb to IIa fibers could have contributed to the increase in GLUT-4 protein content in the muscle of trained individuals. Thus, increased amount of GLUT-4 protein may facilitate augmented cellular uptake of glucose delivered by increased flow to the muscle tissue in the trained individuals.

The increase of GLUT-4 protein was observed in the face of unaltered GLUT-4 mRNA concentration, suggesting that the increase in GLUT-4 is a posttranslational phenomenon. The elevation of GLUT-4 protein could be due to enhanced translation of mRNA and GLUT-4 protein synthesis, or decreased GLUT-4 degradation. The studies were done ≥ 2 d after the last training session. Since there is no increase in GLUT-4 protein content at least when determined immediately after a 3-h exercise period (56) and the training effect on GLUT-4 remains at least for 5 d (16), the elevation of GLUT-4 is probably related to chronic training rather than to the previous acute exercise period. Discordance between changes in muscle GLUT-4 mRNA and protein content has been described in fasted rats (57), which are characterized by hypoinsulinemia. Lower than normal insulin concentrations have also been reported in insulin-sensitive, well-trained athletes both in the basal state and in response to glucose challenge (33, 39). In the current study, serum basal insulin concentrations in the athletes were slightly, although not significantly, below normal. It is probable, however, that in our trained individuals with increased insulin sensitivity the postprandial insulin concentrations would have been lower than normal. If this were the case, the trained individuals would have had a diurnal insulin profile lower than in the sedentary subjects. Thus, a common factor in fasting and training could be relative hypoinsulinemia leading to a posttranslational increase in GLUT-4 protein content.

Regarding the intracellular pathways of increased glucose metabolism, as compared with the sedentary subjects, the athletes had a 62% greater nonoxidative glucose disposal rate and elevated glycogen synthase activity. This resulted in a significant rise in muscle glycogen content during insulin infusion. In addition, there was a significant positive correlation between nonoxidative glucose disposal and glycogen synthase fractional activity. Thus, the greater glucose disposal in the athletes was due to increased glucose storage as glycogen.

Glycogen synthase is the key enzyme in the glycogen synthesis. The glycogen synthase mRNA concentration was similar in the athletes and sedentary individuals. Thus, the rise in glycogen synthase activity in the athletes probably was a posttranslational phenomenon, similar to the rise observed in GLUT-4 protein concentration in the same individuals. We did not determine the enzyme protein concentration. However, total glycogen synthase activity, which should be dependent upon the amount of protein, was not significantly different between athletes and control subjects. This further supports the view that differences in covalent activation of the enzyme rather than variations in the gene expression contributed to elevated glycogen synthase activity in the athletes. Baseline glycogen synthase fractional activity was related inversely to glycogen content and directly to blood flow. These data thus indicate that the lower the muscle glycogen content or the greater the blood flow, the greater the glycogen synthase fractional activity. Regarding a possible causal relationship between glycogen synthase fractional activity and the flow, muscle sample was taken before the flow was measured. Thus, if there is a causal relationship, the time course of these observations suggest that it is muscle glycogen synthase fractional activity that determines the flow, and not the opposite. When insulin infusion began, these associations disappeared and the main determinant of glycogen synthase activity was total glycogen synthase activity, which reflects the protein concentration. These data thus suggest a different regulation of glycogen synthase activity in the basal state and during hyperinsulinemia.

Based on the current data we propose the following mechanism for increased glucose disposal in the athletes. First, physical training leads to increased resting blood flow and glucose and insulin delivery to muscle. Second, GLUT-4 protein concentration in muscle cells is greater in proportion to physical fitness and may facilitate increased glucose uptake into the cell. Third, intracellular glucose metabolism is shifted to the nonoxidative pathway and glycogen synthesis via enhanced basal- and insulin-stimulated activity of glycogen synthase. This mechanism can provide fuel for the muscles at increased rates in trained individuals. There may also be a positive feedback regulation between blood flow and glycogen synthase activity to meet the needs of the muscle. The operation of this mechanism may vary depending on the energy homeostasis, mainly glycogen content, of the muscle tissue.

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