Glucose Uptake in Muscle Cell Cultures from Endurance-Trained Men

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ABSTRACT

BERGGREN, J. R., C. J. TANNER, T. R. KOVES, D. M. MUOIO, and J. A. HOUMARD. Glucose Uptake in Muscle Cell Cultures from Endurance-Trained Men. Med. Sci. Sports Exerc., Vol. 37, No. 4, pp. 579–584, 2005. Purpose: To examine noninsulin- (basal) and insulin-mediated glucose uptake in human skeletal muscle cells from endurance-trained and sedentary individuals. Methods: Muscle biopsies (vastus lateralis) were obtained from competitive, endurance-trained athletes (N = 12; VO2peak 64.9 ± 2.3 mL·kg⁻¹·min⁻¹) and their sedentary counterparts (N = 8; VO2peak 51.8 ± 2.2 mL·kg⁻¹·min⁻¹), and isolated satellite cells allowed to proceed to myotubes. Results: The myotubes exhibited a dose response for glucose uptake with increasing insulin concentrations; maximal glucose uptake was ≈1.5-fold over basal. In relation to exercise training status, basal glucose uptake was significantly (P < 0.05) elevated by ~75% in the endurance-trained versus sedentary men (20.1 ± 2.1 vs 11.9 ± 1.9 pmol·mg protein⁻¹·min⁻¹, respectively). This difference persisted at insulin concentrations of 10 and 1000 μM, although the relative increase in insulin-mediated glucose uptake (fold increase over basal) did not differ between the sedentary and endurance-trained cells. Conclusions: These data suggest that cultured skeletal muscle cells from endurance-trained athletes may differ in respect to basal glucose uptake. Key Words: EXERCISE TRAINING, GLUCOSE TRANSPORT, INSULIN SENSITIVITY, MYOTUBES

Skeletal muscle cell cultures from animal and human species have been used to characterize and study glucose uptake (14). A common model is to utilize human skeletal muscle cells (HSMC) that are derived from precursor satellite cells located between the basal lamina and the plasma membrane of muscle fibers. Briefly, the satellite cells are isolated and treated in culture to form proliferative, mononucleated myoblasts (8,13,14). After additional manipulations, the myoblasts cease proliferation and fuse into multinucleated myotubes (8,13,14). Myotubes from human skeletal muscle are insulin responsive and exhibit morphological and chemical characteristics of intact skeletal muscle fibers (8,13–15,19).

Skeletal muscle is the primary site for insulin-mediated glucose disposal (4); HSMC has thus been utilized when studying mechanisms involved with the insulin-resistant state. For example, myotubes from patients with Type 2 diabetes mellitus were insulin resistant compared with nondiabetic controls (3,8,9). Myoblasts from the first-degree relatives of Type 2 diabetics also displayed insulin resistance (11), as did myoblasts from insulin-resistant Pima Indians (22,24). These findings suggest that some facet of in vivo insulin resistance can remain expressed in HSMC. The explanation of how in vivo insulin resistance induces the precursor satellite cells to remain insulin resistant in HSMC, however, remains elusive.

Surprisingly, there has been relatively little examination of the characteristics of HSMC in tissue samples from individuals with relatively high rates of noninsulin or insulin-mediated glucose transport. Endurance-oriented exercise training consistently enhances whole-body insulin action and noninsulin-mediated glucose uptake (7,23); however, it is not known if these characteristics are maintained in HSMC. The purpose of the current study was to therefore use a cross-sectional design and compare basal and insulin-stimulated glucose uptake rates in myotubes raised in culture from the skeletal muscle of endurance-trained athletes versus sedentary subjects. We hypothesized that insulin-stimulated glucose transport in HSMC would not differ with training because of the acute nature of the exercise response (8).

METHODS

Experimental design. Glucose uptake rate was compared in HSMC from 12 endurance-trained athletes and 8 sedentary male subjects. Participants were categorized based on reported physical activity and participation as intercollegiate athletes in sports requiring run training (track and cross country). The rationale for selecting intercollegiate athletes was to maximize the exposure of the muscle to contractile activity. Sedentary participants reported no struc-
tured exercise for the previous 12 months. Participants were not taking any medications or ergogenic aids known to alter carbohydrate metabolism. Additionally, as indicated by a health history questionnaire, participants reported no family history (blood relatives) of diabetes. Before testing, approval was given by the East Carolina University Policy and Review Committee on Human Research. Participants signed an informed consent as approved by the East Carolina University Policy and Review Committee on Human Research.

**Muscle biopsy and plasma.** Skeletal muscle (50–100 mg) was obtained from the vastus lateralis using the percutaneous needle biopsy technique (5) after a 12-h overnight fast. Trained participants were instructed to perform their regular exercise routine the day before the biopsy. A fasting venous blood sample was taken immediately before the biopsy. Plasma was separated and frozen at −80°C for subsequent analyses. Plasma was analyzed for glucose (YSI 2300 STAT Plus Glucose and Lactate Analyzer, YSI Inc., Yellow Springs, OH) and by microparticle enzyme immunoassay for insulin (IMx, Abbott Laboratories, Abbott Park, IL). Values were used to determine homeostasis model assessment (HOMA) values (fasting glucose (mg·dL−1) × 0.05551 × fasting insulin (μU·mL−1)/22.1) as an index of in vivo insulin action (16).

**Exercise testing.** Peak oxygen consumption was determined during a graded treadmill test. Initial speed was based on training status (8 and 5 miles-h−1 for the trained and sedentary subjects, respectively). Treadmill grade and/or speed were increased every 2 min until voluntary exhaustion was achieved. The protocol was designed to achieve fatigue within 8–12 min. Heart rate and expired gases (TrueMax 2400, Consentius Technologies, Sandy, UT) were monitored continuously. Criteria for a successful maximal test included achieving at least three of the following: 85% of age-predicted (220 − age) maximal heart rate, voluntary exhaustion, a respiratory exchange ratio of >1.10, or a failure of oxygen uptake to increase with an increased workload. An activity survey provided information on the mode and time completed for various forms of physical activity (i.e., walking and running); energy expenditure upon physical activity was estimated (17). Body composition was determined with seven-site skinfolds (12).

**HSMC materials.** 2-deoxy-[^3H] glucose (2-DG) was obtained from New England Nuclear Life Sciences Products (Boston, MA). Bovine serum albumin (Fraction V), porcine insulin, EDTA, and HEPES sodium salt were from Sigma-Aldrich (St. Louis, MO). Heat-inactivated bovine serum (FBS), heat-inactivated horse serum (HS), Ca2+ and Mg2+ free Hank’s balanced salt solution (HBSS), Type IV collagenase, lymphophilated trypsin, and trypsin-EDTA subculturing cocktail were from Gibco (Grand Island, NY). Growth media (SkGM) and differentiation media (SkDM) consisted of low-glucose (5 mM) Dulbecco’s modified eagle medium (DMEM) from Gibco, supplemented with human skeletal muscle SingleQuots® from Clonetics (San Diego, CA). Biocoat® tissue culture T-25 flasks and 24-well plates were from Becton Dickenson (Franklin Lakes, NJ). The bicinchoninic acid (BCA) protein assay kit was from Pierce (Rockford, IL).

**Culture procedure.** The harvesting and subsequent culturing of satellite cells from skeletal muscle biopsy material was adapted from Henry et al. (9), with modifications as indicated. Biopsied muscle was immediately placed in ice-cold DMEM and weighed. The tissue was then dissected free of adipose and connective tissue in ~3 mL DMEM + ~5 mL HBBS at room temperature. The tissue was minced (2-cm pieces) and washed twice by centrifugation for 10 min with 20 mL of Hanks at 550 × g (Beckman TJ-6). Satellite cells were isolated with a trypsin digestion cocktail containing 0.25% (wt/v) trypsin, 0.1% (wt/v) Type IV collagenase, and 0.1% (wt/v) BSA for 30 min on a low shaker setting at room temperature. Digestion was terminated with 5% FBS (wt/v), and cells were preplated in uncoated 25-cm² flasks for 1–3 h at 37°C to remove fibroblasts. This protocol has been shown to be effective at minimizing fibroblast contamination. The residual cellular suspension was transferred to collagen I–coated 25-cm² flasks in 3 mL of SkGM (DMEM supplemented with 10% FBS, 0.5 mg·mL−1 BSA, 0.5 mg·mL−1 fetuin, 20 ng·mL−1 human epidermal growth factor, 0.39 μg·mL−1 dexamethasone, and 50 μg·mL−1 gentamicin/amphotericin B), and incubated in a 5% CO2, 37°C humidified atmosphere. To speed myoblast attachment to the collagen matrix, the volume of SkGM was increased to 5 mL after 24 h of incubation. The number of attached myoblasts was counted after 5 d of incubation immediately after the first media change. This represented the initial number of viable myoblasts for determining population doublings, and ranged from 60 to 120 cells·cm⁻². Every 5 d, SkGM was changed until the cultures reached ~80% confluence (approximately 4–5 wk). Myoblasts were counted and subcultured into 24-well plates at a cell density of 30,000 cells per well for glucose transport experiments. The number of population doublings was estimated between 10 and 13 using a previously published formula (22). After cell attachment and 90% confluence (2–3 d), differentiation was induced by switching the media to SkDM (DMEM supplemented with 2% HS, 0.5 mg·mL−1 BSA, 0.5 mg·mL−1 fetuin, and 50 μg·mL−1 gentamicin/amphotericin B) and continuing to incubate as before with media changes every 2 d. Glucose uptake was measured in 8-d differentiated myotubes; in preliminary experiments (18), we determined that this differentiation period resulted in the optimal rate of glucose uptake. At 8 d, there was also myosin protein expression (Western blot) and visual confirmation of multinucleated myotubes, indicating differentiation. A representative photograph of the change from confluent myoblasts to multinucleated myotubes is presented in Figure 1 along with myosin protein content at various time points. Although glucose uptake and protein expression after 8 d of differentiation were not significantly different from 3 d of differentiation, cells were differentiated for 8 d to account for the possibility that some cultures may require a longer differentiation period to reach the same degree of multinucleation. After the differentiation time period, cultures were visually inspected to verify myotube formation. Additionally, we have reported that this cell
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**Results**

**Subjects.** Subject characteristics are presented in Table 1. The endurance-trained men possessed a high aerobic capacity ($V_{O2peak}$) similar to that reported in other male endurance-trained athletes (1,10,23), had a lower percentage of body fat, and expended more calories per week on exercise than the sedentary group. There was a trend for fasting insulin ($P = 0.07$) and HOMA ($P = 0.12$) to be lower (indicating enhanced insulin action) in the endurance-trained group (Table 1). These data are similar to others (21) where fasting insulin concentration was not markedly different between trained and untrained young individuals, yet insulin action as determined by a euglycemic/hyperinsulinemic clamp was dramatically greater in the physically active subjects.

**Glucose uptake in cultured myotubes.** Data from both groups were pooled ($N = 20$) to determine whether insulin increased glucose uptake. Insulin significantly increased ($P < 0.05$) glucose uptake over basal at $10 \mu M$ ($\sim$13-fold) and $1000 \mu M$ ($\sim$1.5-fold), indicating the cells were insulin responsive.

Data comparing glucose uptake in myotubes from sedentary and endurance-trained men are presented in Figure 2. Absolute glucose uptake (pmol·mg$^{-1}$·protein·min$^{-1}$) was significantly higher in myotubes from the endurance-trained men in the absence of insulin (basal) and at both insulin concentrations. The difference in glucose uptake was approximately $9$ pmol·mg$^{-1}$·protein·min$^{-1}$ ($\sim$75%). As presented in Figure 2, the difference in basal glucose uptake accounted for the difference in insulin-stimulated glucose uptake between the sedentary and trained individuals; accordingly, the fold stimulation over basal between the sedentary and trained subjects at each insulin concentration did not differ significantly between the two groups.

**Statistical analysis.** Values are expressed as mean ± standard error (SE). Comparison of subject characteristics was performed with a Student’s $t$-test. A $1 \times 3$ repeated measures analysis of variance (ANOVA) was used to determine whether insulin stimulated glucose uptake over basal regardless of training status. A $2 \times 3$ repeated measures ANOVA was used to determine whether varying concentrations of insulin altered glucose uptake rate in the trained versus sedentary individuals. Significance was set at $P < 0.05$.

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**Table 1. Characteristics of the sedentary ($N = 8$) and endurance-trained ($N = 12$) men.**

<table>
<thead>
<tr>
<th></th>
<th>Sedentary</th>
<th>Endurance Trained</th>
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<tbody>
<tr>
<td>Age (yr)</td>
<td>23.4 ± 1.0</td>
<td>23.0 ± 1.4</td>
</tr>
<tr>
<td>Stature (m)</td>
<td>1.74 ± 0.0</td>
<td>1.74 ± 0.0</td>
</tr>
<tr>
<td>Mass (kg)</td>
<td>75.48 ± 4.7</td>
<td>69.72 ± 1.3</td>
</tr>
<tr>
<td>Body mass index (kg·m$^{-2}$)</td>
<td>24.66 ± 0.9</td>
<td>22.89 ± 0.5</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>11.28 ± 2.1</td>
<td>7.03 ± 0.9*</td>
</tr>
<tr>
<td>$VO_{2peak}$ (L·min$^{-1}$)</td>
<td>3.96 ± 0.2</td>
<td>4.55 ± 0.2*</td>
</tr>
<tr>
<td>$V_{O2peak}$ (mL·kg$^{-1}$·min$^{-1}$)</td>
<td>51.80 ± 2.2</td>
<td>64.98 ± 2.3*</td>
</tr>
<tr>
<td>Weekly exercise (kcal)</td>
<td>63.0 ± 50.0</td>
<td>3246.0 ± 628*</td>
</tr>
<tr>
<td>Fasting insulin (µU·mL$^{-1}$)</td>
<td>5.95 ± 1.2</td>
<td>4.21 ± 0.5</td>
</tr>
<tr>
<td>Fasting glucose (mg·dL$^{-1}$)</td>
<td>87.61 ± 1.7</td>
<td>90.02 ± 3.1</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.29 ± 0.3</td>
<td>0.97 ± 0.2</td>
</tr>
</tbody>
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Mean ± SE; HOMA: homeostasis model assessment. * $P < 0.05$ for comparison between groups.
not significantly differ. There were no statistically significant relationships between the characteristics of the subjects (Table 1) with variables measured in HSMC (Fig. 2).

**Real-time quantitative PCR.** Based on the observation of increased basal glucose uptake in trained individuals, three endurance-trained cross-country runners (age $= 22.0 \pm 1.5$ yr, BMI $= 21.2 \pm 0.7$ kg·m$^{-2}$) and four sedentary (age $= 29.2 \pm 1.1$ yr, BMI $= 26.3 \pm 1.4$ kg·m$^{-2}$) underwent an additional biopsy for the quantification of GLUT1 gene expression in HSMC cultures after differentiation into myotubes. Briefly, total RNA was isolated from cells using an RNeasy Mini Kit purchased from QIAGEN, Inc. (Valencia, CA). Although this technology selectively binds RNA to a silica-gel membrane, an oncolumn DNase digestion was performed using RNase-Free DNase Set, also purchased from QIAGEN, Inc., to remove any residual DNA that may be present in the sample. After isolation, RNA was quantified using the RiboGreen® RNA quantitation kit (Molecular Probes, Eugene, OR) and reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). RTQ-PCR was conducted using an ABI PRISM 7700 sequence detection system with the primer/probe sets designed using ABI software from the sequence obtained in the GenBank database. The sequence for the GLUT1 gene was: F: GCGGAATTCAATGCTGATGAT, R: CAGTTTCGAGAAGCCCATGAG, P: CTGGCCTTCGTGTCGCCGCT. Relative quantification of the target gene was determined as previously described (18). The relative difference in human GLUT1 gene expression is presented in Figure 3. Although not statistically different ($P = 0.14$), there was a trend for higher GLUT1 expression in the trained individuals.

**DISCUSSION**

Exercise-induced alterations in glucose uptake are relatively short lived. For example, insulin-mediated glucose uptake has been reported to return to control levels in endurance athletes, similar to those in the present study, after as little as 7–14 d of training cessation (1,9). Also, enhanced contractile-stimulated glucose uptake rapidly returns to noncontractile levels after cessation of the exercise bout (12). With the HSMC procedure, the cells are incubated/differentiated for approximately 4–5 wk after extraction of the tissue; it would be logical to assume that the training- and exercise-induced alterations in glucose transport would be lost after such a prolonged absence of contractile activity. Based upon such information, we therefore hypothesized that any differences in insulin action evident *in vivo* would not be retained in skeletal muscle cells raised in culture. This hypothesis was confirmed in the present study, as the relative stimulation in glucose uptake with insulin exposure did not differ in myotubes derived from endurance-trained athletes compared with sedentary individuals (Fig. 2), despite evidence that the trained men were more insulin sensitive (lower fasting plasma insulin concentrations, $P = 0.07$, Table 1).

Although there were no differences in insulin-stimulated glucose uptake, we did observe that basal (noninsulin-mediated) glucose uptake was enhanced in myotubes cultured from the skeletal muscle (vastus lateralis) of endurance-trained athletes (Fig. 2). We initially tested 6 subjects in each group and obtained this result; to validate the finding, we added additional men to obtain 12 and 8 subjects for the trained and untrained groups, respectively. This observation of an increase in noninsulin-mediated glucose uptake with endurance training is in agreement with other *in vivo* data. Using a cross-sectional study design similar to the present

![Figure 2](http://www.acsm-msse.org)

**FIGURE 2**—Absolute (A) and relative (B) (fold-increase over basal) basal and insulin-mediated glucose uptake in myotubes from exercise-trained ($N = 12$) and sedentary ($N = 8$) men. *Significantly elevated at the respective insulin concentration compared with the other group, $P < 0.05$.

![Figure 3](http://www.acsm-msse.org)

**FIGURE 3**—Relative difference in human GLUT1 gene expression between exercise-trained ($N = 3$) and sedentary ($N = 4$) men determined by RTQ-PCR.
contractile activity may actively imprint satellite cells in the satellite cell compartment (2). The present findings suggest that some aspect of proliferation (2). When these animals were treated with insulin-like growth factor one (IGF-1) to increase basal glucose uptake in skeletal muscle and explain our (Fig. 2) and other (23) findings. Therefore, as a follow-up experiment cells from trained and sedentary subjects were used for RNA isolation and subsequent determination of GLUT1 gene expression in differentiated myotubes. Although differences did not reach statistical significance, there was a trend for trained subjects to have higher GLUT1 expression (Fig. 3). This observation is similar to findings of Henry et al. (8), who have reported decreased GLUT1 content in cell cultures obtained from diabetic subjects who displayed reduced basal glucose uptake (8). It should be noted there is a discrepancy regarding the role of GLUT1 in adult skeletal muscle. Although GLUT1 has been reported in adult muscle (19), Gaster et al. (6) have reported that it is only important in developing skeletal muscle and almost absent in adults. However, it has been determined that GLUT1 plays a pivotal role in basal glucose uptake in HSMC cultures (8). Further studies are needed to characterize how exercise training may regulate GLUT1 gene expression and protein content in HSMC.

Although an increase in noninsulin-mediated glucose uptake with endurance training has been reported in vivo (23), our finding of a difference between exercise-trained and sedentary individuals in the HSMC model (Fig. 2) is somewhat surprising, because of the acute nature of the exercise response in relation to glucose uptake. Also, it is not evident why any aspect of exercise training/contractile activity would be retained in the noncontractile satellite cells that are used in HSMC for establishing the primary culture systems. Nevertheless, some findings suggest that satellite cells may be imprinted with the current environmental conditions of the tissue donor. For example, animals subjected to repeated bouts of immobilization have decreased in vitro satellite cell proliferation (2). When these animals were treated with insulin-like growth factor one (IGF-1) in vivo, satellite cell proliferation in vitro was restored, demonstrating cell plasticity (2). The present findings suggest that some aspect of contractile activity may actively imprint satellite cells in skeletal muscle in manner producing enhanced basal glucose uptake; this adaptation is then retained and expressed after repeated cell divisions and differentiation into myotubes despite the absence of contractile activity.

Other HSMC data suggest that findings from primary skeletal muscle cells reflect inherent characteristics present in the donor. For example, the finding of a reduced rate of insulin-stimulated glucose transport in HSMC from Type 2 diabetics and their kin prompted the suggestion that Type 2 diabetes is primarily of a genetic origin (7,22). The current study examined the effect of exercise training using a cross-sectional design. It is plausible that inherent genetic characteristics of the endurance-trained athletes could account for the differences in noninsulin-mediated glucose uptake we observed. However, as we did not specifically explore mechanistic differences, the cellular alteration responsible for the enhancement in basal glucose uptake with endurance training remains to be elucidated. Regardless, the potential for differences in HSMC due to some aspect of endurance training is intriguing and should be validated and studied.

The properties that characterize HSMC as a viable model for studying glucose transport have been reported by others (3,8,9,13,15,19). As in the current study (Fig. 2), myotubes from human skeletal muscle exhibit a dose-response for glucose uptake with increasing insulin concentrations (8,15,19). Functioning insulin receptors and components of the insulin-signaling pathway have also been identified in HSMC myotubes (3,8,15). A positive characteristic of HSMC is that detectable levels of the insulin-sensitive (GLUT4) and noninsulin-sensitive (GLUT1) glucose transporters are evident (3,8,19).

In summary, myotubes that were cultured from skeletal muscle biopsies obtained from endurance-trained athletes did not differ in insulin-stimulated glucose uptake. However, the cells from the endurance-trained individuals displayed elevated basal, noninsulin-mediated glucose transport compared with their sedentary counterparts. These data suggest that cultured skeletal muscle cells from endurance-trained athletes may differ in respect to basal glucose uptake.

Special thanks for the contributions of G. Lynis Dohm, Robert Hickner, Len Klepack, Mike Van Scott, and Jack Youngren to this research.

The experiments were supported by grant DK56112 (Houmard).

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