Exercise training favors increased insulin-stimulated glucose uptake in skeletal muscle in contrast to adipose tissue: a randomized study using FDG PET imaging

M. H. Reichkendler,1,2 P. Auerbach,1 M. Rosenkilde,1 A. N. Christensen,2,5 S. Holm,2 M. B. Petersen,1 A. Lagerberg,1 H. B. W. Larsson,4 E. Rostrup,4 T. H. Mosbech,5 A. Sjödin,3 A. Kjaer,1,2 T. Ploug,1 L. Hoeggaard,2 and B. Stallknecht1

1Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; 2Department of Clinical Physiology, Nuclear Medicine and PET, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; 3Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark; 4Functional Imaging Unit, Glostrup Hospital, University of Copenhagen, Copenhagen, Denmark; and 5Department of Informatics and Mathematical Modeling, Technical University of Denmark, Copenhagen, Denmark

We have previously shown that 11 wk of moderate- (~30 min/day) and high-dose (~60 min/day) aerobic physical exercise increases peripheral insulin sensitivity to the same extend in overweight men as measured by the hyperinsulinemic-isoglycemic clamp technique (31). However, it was not addressed in which tissues the increase occurred. Skeletal muscle (constituting ~40% of body mass) is the major tissue involved in the glucose metabolism and an important site of insulin resistance in obesity and type 2 diabetes (1). Insulin resistance is associated with a reduced percentage of red oxidative skeletal muscle fibers (10), as glucose uptake capacity is larger in red oxidative than in white glycolytic muscle fibers (12, 17). Skeletal muscle shows regional heterogeneity metabolically and by distribution of fiber types, with the same tissue at different locations having different metabolic and structural properties (13, 23). This metabolic heterogeneity is also found in adipose tissue (8).

Physical training is well known to increase insulin-stimulated glucose uptake in the leg as measured by Fick’s principle (5, 6). However, this technique does not allow for differentiation between glucose uptake in various tissues of the leg or between individual muscle groups. Uptake of glucose in different tissues can be estimated noninvasively by use of computer tomography (CT) and PET using the glucose analog tracer 2-deoxy-2-[18F]fluoro-D-glucose (FDG) (43). The effect of endurance training on insulin-stimulated femoral skeletal muscle glucose uptake has been evaluated by FDG PET in two cross-sectional studies, in which glucose uptake was found to be higher in endurance-trained men than in resistance-trained and sedentary men (21, 39), but no randomized controlled trials have addressed the issue. Regional subcutaneous adipose tissue (SAT) glucose uptake in relation to physical activity/inactivity has previously been investigated with the microdialysis and 133Xe washout techniques (7, 9, 15, 37, 38), but these techniques cannot be used to estimate metabolism of visceral adipose tissue (VAT) in humans.

Therefore, the aim of the present study was, in a randomized controlled design, to investigate the effect of 11 wk of moderate- or high-dose aerobic physical exercise on insulin-stimulated glucose uptake in individual femoral muscle groups, intra- and retroperitoneal VAT, and abdominal (both anterior and posterior) and femoral SAT in sedentary, young, and moderately overweight men using the noninvasive FDG PET/CT methodology. Furthermore, changes in leg fat and fat-free masses (FFM) and in abdominal SAT and VAT masses were...
assessed along with the protein expression of glucose transporter-4 (GLUT4) in femoral muscle.

MATERIALS AND METHODS

Study design. The study design, participant criteria, and exercise regimen have been described in detail previously (31, 32). In short, subjects eligible for inclusion were Caucasian males, 20–40 yr old, moderately overweight (BMI 25–30 kg/m², %body fat ≥25%), sedentary [not engaged in regular exercise, maximal oxygen consumption (VO₂max) ≤45 ml O₂/kg body mass/min], healthy (according to interview and fasting blood glucose <6.1 mmol/l, blood pressure <140/90 mmHg, no regular medication), weight stable (±2 kg during the preceding 6 mo), with no type 2 diabetic first-degree relatives and willing to adhere to the protocol (31). The subjects were randomized to a control group (CON), a moderate-dose training group (MOD), or a high-dose training group (HIGH). After a 3-wk pretesting period, the subjects continued their sedentary lifestyle (CON) or performed daily aerobic exercise of 300 kcal/day (MOD) or 600 kcal/day (HIGH) for 11 wk. Three times per week, exercise sessions were more intense (>70% of VO₂max). For the remaining sessions, an intensity of 50–70% of VO₂max was recommended. All sessions were monitored using heart rate monitors (RS400; Polar Electro OY, Kempele, Finland). The posttesting period began after ~10 wk of intervention, and the subjects adhered to protocol during this period.

The data presented are part of a larger study on metabolic and cultural health in moderately overweight men [Project Four-IN-onE (FINE); http://fineku.dk]. The study adhered to the Helsinki II declaration, was approved by The Ethics Committee of Copenhagen (H-4-2009-089), and was registered at http://www.clinicaltrials.gov/ with the identifying code NCT 01430143. Subjects signed informed consent after receiving detailed oral and written information regarding the study. Withdrawal of informed consent or insufficient training compliance resulted in exclusion from the study (31).

Tests. Participants completed three separate test days at baseline and at the end of the intervention. On the first test day, body composition was assessed by dual-energy X-ray absorptiometry (DEXA) scanning. On the second test day, participants underwent a hyperinsulinemic-isoglycemic clamp, had biopsies taken from skeletal muscle, and had FDG PET/CT scans performed. On the third test day, an MRI scan was performed. The two first test days were performed ~1 wk apart after a 12-h overnight fast; the MRI scan was performed in the afternoon after an 8-h fast. For 36 h prior to the two first test days, the participants had been instructed not to engage in physical exercise or any other strenuous physical activities, and they transported themselves by car or public transportation to the laboratory.

Body composition. Body weight and height were measured (SECA, Birmingham, UK). Body composition was assessed by DEXA scanning (Prodigy Bone Densitometer System; GE Lunar, Madison, WI), including leg fat mass and fat free mass (FFM), each leg delimited as a region of interest (ROI) by the inguinal ligament.

MRI scans were performed on a 3T Philips Achieva scanner system (Philips Medical Systems, The Netherlands) using a 16-element phased array coil (SENSE XL Torso coil; Philips Medical Systems) with the subjects in the supine position. Axial T1-weighted MRI images of the abdomen were obtained using a turbo spin echo sequence (36 slices, 7 mm thickness, 1 mm interslice distance, time repetition = 500 ms, time echo = 15 ms, flip angle 90°). Images from the pre- and postintervention examinations were carefully matched, and images were delimited by the T11–12 disc and the L4–5 disc and segmented using an automated procedure developed by Mosbech et al. (19). Slices not properly segmented automatically were manually segmented using SlicerOMatic (v. 4.3, 2006; Tomovision, Montreal, QC, Canada) by an investigator blinded to the identity of the subjects. Volumes of abdominal VAT and SAT were calculated using the two-column model (35), and an adipose tissue density of 0.92 g/ml (42) was used in the conversion from volume to mass.

Hyperinsulinemic-isoglycemic clamp. Participants were subjected to a hyperinsulinemic-isoglycemic clamp with an insulin infusion of 40 mU·min⁻¹·m⁻² in order to determine peripheral insulin sensitivity (4a). Biopsies of skeletal muscle (vastus lateralis) were obtained at baseline and after 120 min of clamp. The procedures for the hyperinsulinemic-isoglycemic clamp, obtaining biopsies, Western blotting, blood sampling, and calculation of peripheral insulin sensitivity have been described in detail previously (31). The primary antibody used for Western blotting of muscle homogenates was a mouse monoclonal antibody (F27) raised against a peptide corresponding to the 13 COOH-terminal amino acids of GLUT4. The clamp was maintained during biopsy and throughout the PET/CT scans. Due to logistic reasons, blood sampling during the PET/CT scan (for adjusting the clamp and to calculate AUC from 60–120 min after injection of FDG) was performed only once every 10 min, and plasma insulin levels were not measured. The calculation of peripheral insulin sensitivity/whole body glucose uptake at this time (approximately from 4 to 5 h after clamp start) was therefore not adjusted for plasma insulin concentration. To compare total body glucose uptake with regional glucose uptake in the individual tissues, total body glucose disposal rate (Rg) in micromoles per minute was calculated for the first 60 min after FDG injection as

\[ R_g = \frac{(1,000 \text{ mmol}\text{/mol} \times \text{GIR}_{\text{PET}0-60})}{\text{TBM}} \]

where GIRPET 0–60 is the average glucose infusion rate from 0–60 min after FDG injection (mg·min⁻¹·kg total body mass⁻¹), TBM is the total body mass (kg), and M is the molar mass of glucose (180.16 g/mol).

PET/CT scans: tracer, image acquisition, and processing. PET/CT scans were performed on a Siemens Biograph 40 or 64 slice PET/CT (Siemens, Erlangen, Germany). To minimize subject movement, subjects were placed comfortably in a supine position with a large vacuum pillow placed around their legs. Injected FDG activity target was 200 MBq, and total effective dose throughout the study was <10 mSV. The scanning protocol for PET/CT consisted of a 60-min dynamic scan of the femoral region delimited cranially below the scrotum followed by a 15-min scan of the brain (data not shown) and a 4-min per bed scan of the thorax and abdomen. The time from FDG injection to the start of static scans of brain and thorax/abdomen was for each subject similar at pre-[abdominal scan: 100(8) min] and post-[abdominal scan: 102(9) min] intervention. An ultra-low-dose CT (120 kV, 11 mA) for attenuation correction and anatomic localization of the adipose tissue and skeletal muscle was performed directly prior to each scan. PET scans were acquired in List Mode, and the femoral scan was subsequently histogrammed into 26 time frames (12 × 10s, 4 × 120s, 10 × 300s) and reconstructed using the Ordered Subset Expectation Maximisation Point Spread Function correcting method (TrueX, Siemens), 336 × 336 pixels, with 8 iterations and 21 subsets with 4-mm Gaussian smoothing. The thoracic-abdominal scan was reconstructed as a single time frame with 336 × 336 pixels, 3 iterations, 21 subsets and 2-mm Gaussian smoothing. All PET images were corrected for attenuation and scatter, and decay corrected to scan start.

Image analysis. All PET image analyses were performed using PMOD 3.304 (PMOD Technologies, Zurich, Switzerland) by an investigator blinded to the subject intervention allocation. The femoral and abdominal CT scans were initially filtered using the 3-mm Median Smooth 3D function.

Femoral PET/CT scan. Volumes of interests (VOI) in skeletal muscle and SAT were automatically segmented for each leg using the Connected Threshold ITK segmentation function on slice 10–101 of 111, with a subsequent erosion of three pixels from the borders of each region to minimize partial volume effects (Fig. 1B). Following this, slices 40–50 were averaged, and ROIs were manually drawn on the filtered CT image bilaterally for five individual muscles or muscle groups: vastus medialis, vastus lateralis, the hamstring muscle group, the adductor muscle group, and the gracilis muscle (Fig. 1C). The PET image was subsequently evaluated to ensure that the femoral artery...
was not in close proximity to any ROI. For each VOI and ROI, a full kinetic analysis was performed, i.e., a determination of the four rate constants in the standard two-tissue model for FDG (36). An image-derived input function (IDIF) from the femoral artery was used as previously described (24). This method was chosen after comparison with other suggested IDIF methods (4) using arterial blood samples obtained from 24 scans on 14 persons as the gold standard for validation of the different methods. Using the method by Parker et al. (24), we found a consistent underestimation, which could be rectified by a calibration using a linear transformation. When plotting the \( K_m \) values calculated based on blood sampling and those calculated with the Parker method by linear regression a slope of \( \frac{y}{x} = 0.82 \), and a zero intercept with \( R^2 = 0.94 \) was obtained (data not shown). Since linear regression is unbiased, we can divide the \( K_m \) values obtained by the Parker method with the slope and get unbiased results. This gives a small increase in variance, which was estimated by bootstrap and cross-validation to be 0.0017 min\(^{-1}\), i.e., \( \pm 5\% \).

Metabolic rate of glucose (MR\(_{\text{glu}}\)) was calculated as

\[
\text{MR}_{\text{glu}} = \frac{(1,000 \times K_m \times C_{bg})(LC \times \rho)}{A \times m/d}
\]

where \( K_m \) is the rate constant for FDG (ml blood/ml tissue \(^{-1}\)min\(^{-1}\)), \( C_{bg} \) is the blood glucose concentration (mmol/l), \( LC \) is the lumped constant describing the different properties of FDG and glucose for transport and phosphorylation/dephosphorylation and \( \rho \) is the tissue density. Values of 1.14 and 1.20 were used for adipose tissue and skeletal muscle LC, respectively (28, 43), and values of 0.92 (42) and 1.06 (18) g/ml were used for adipose tissue and skeletal muscle density, respectively.

Abdominal PET/CT scan. VOIs of abdominal (anterior and posterior) SAT as well as intraperitoneal and retroperitoneal VAT were automatically segmented using the threshold connected segmentation function with a Hounsfield unit range of 150 to 50 on manually predefined cubic and spheric volumes between T11–12 and L5/S1 (Fig. 1A). A three-pixel erosion was performed on each VOI to minimize spill-in/spill-over effects from surrounding tissue (so-called partial volume effects). Standardized uptake value (SUV\(_{\text{mean}}\); g/ml) for each VOI was calculated as

\[
\text{SUV}_{\text{mean}} = \frac{\text{A}}{\text{m} / \text{d}}
\]

with A being the mean activity concentration of a VOI (kBq/ml), \( m \) being the total body mass of the subject (kg), and d being the injected dose of FDG (MBq). All activities were decay-corrected to scan start.

For the abdominal VAT and SAT, we estimated a value, eMR\(_{\text{glu}}\) in VOIs by using the area under curve (AUC) of the input function and the relation

\[
e\text{MR}_{\text{glu}} = \frac{(1,000 \times A \times C_{bg})}{(\text{AUC} \times LC \times \rho)}
\]

where AUC is the area under the curve of the input function based on the IDIF from 0–60 min and arterialized venous blood samples from 60–120 min. \( K_m \) for the abdominal scan was thereby estimated as.
Glucose uptake in different tissue depots. The total glucose uptake rate per tissue depot (μmol/min) pre- and postintervention for the depots of skeletal muscle, femoral adipose tissue (AT), and abdominal VAT and SAT was estimated as

\[ \text{MR}_{\text{glu(total)}} = m \times \text{MR}_{\text{glu(tissue)}} \]

where \( m \) is the mass of the specific tissue depot (kg) from MRI (abdominal VAT and SAT) or DEXA scan (skeletal muscle mass, total fat body mass, leg fat mass), and \( \text{MR}_{\text{glu(tissue)}} \) is the glucose uptake rate of the same tissue depot (μmol·kg\(^{-1}\)·min\(^{-1}\)). Skeletal muscle was assumed to constitute 54% of FFM estimated from DEXA scan (45). It was further assumed that all skeletal muscle had the metabolic properties of femoral muscle, which was therefore used for the skeletal muscle depot calculation. It was also assumed that all leg fat has the metabolic properties of femoral SAT. For abdominal SAT, a mean of anterior and posterior abdominal SAT \( \text{eMR}_{\text{glu}} \) was used, and similarly for abdominal VAT, a mean of intra- and retroperitoneal \( \text{eMR}_{\text{glu}} \) was used.

Statistical analysis. The study was primarily an efficacy study, and results are presented as per-protocol analysis. Within-group differences were tested using a paired \( t \)-test. An analysis of covariance (ANCOVA) with group baseline values and group assignment as specified covariates with ad hoc adjustment using the Tukey procedure was used to assess changes from pre- to postintervention between groups. Results are given as the average of individual bilateral mean and expressed as either pre- or post-mean values (SD) or as \( \Delta \) value (CI 95%) for within-group changes. Compared with CON using the

Fig. 2. Flow chart of subjects and completed tests. CON, sedentary control group; MOD, moderate-dose exercise group; HIGH, High-dose exercise group; Pre, baseline; Post, after 11-wk intervention.
E500  DIFFERENTIAL EFFECTS OF EXERCISE ON REGIONAL GLUCOSE UPTAKE

ANCOVA, results are expressed as least square means (LSM; CI 95%) of the difference between the exercise groups (MOD or HIGH) vs. CON. Associations between either baseline or Δ values (post-/preintervention) were determined using Pearson product correlation. Data not normally distributed were log transformed. Participants violating the fasting and/or exercise instructions prior to a test day were excluded from the data analysis of the test day in question. A level of $P \leq 0.05$ was considered significant.

Power and sample size calculations were performed prior to the study and were based on changes in peripheral insulin sensitivity. To obtain a statistical power of 80%, 3 × 17 persons were necessary to detect a 33% increase in glucose uptake in intervention groups compared with CON with a CI of 83% and an α-level of 0.05. Statistical analysis was conducted in SAS Enterprise Guide 4.2 (SAS Institute, Cary, NC).

RESULTS

Subjects. Sixty-one subjects were randomized to three groups, and eight subjects were excluded during the intervention (Fig. 2). Thus, the number of subjects completing the intervention was 17 in CON [31(6) yr], 18 in MOD [30(7) yr], and 18 in HIGH [28(5) yr] ($P > 0.1$). At preintervention, average duration of the hyperinsulinemic-isoglycemic clamp before injection of FDG was 241(22) min and the injected dose of FDG was 207(9) MBq. At postintervention, the duration of the clamp prior to FDG injection was 248(21) min and the injected FDG dose was 203(11) MBq. No differences were observed between pre- and post-values compared with CON for either average clamp duration before FDG injection or injected FDG dose ($P > 0.1$).

Skeletal muscle glucose uptake. Whole body insulin sensitivity increased 28% in MOD and 36% in HIGH, as previously reported (31). Femoral skeletal muscle $MR_{glu}$ increased within group in both intervention groups ($P = 0.003$; MOD 26%, HIGH 31%), but only a trend remained in HIGH compared with CON ($P = 0.08$) while still significant in MOD ($P = 0.005$; Fig. 3A). At baseline, whole body insulin sensitivity at 0–60 min after FDG injection (Table 1) correlated with femoral skeletal muscle $MR_{glu}$ ($r = 0.63, P < 0.0001$). However, no correlation was found for intervention-induced changes ($P > 0.1$).

The $MR_{glu}$ of five individual femoral muscles (vastus medialis, vastus lateralis, the hamstring muscle group, the adductor muscle group, and gracilis) increased within group in both MOD (all $P < 0.01$) and HIGH (all $P < 0.05$) (Fig. 3B–F). Compared with CON, the increase was significant in MOD for vastus medialis, vastus lateralis, and the hamstring group. The correlation between whole body insulin sensitivity at 0–60 min after FDG injection and glucose uptake of the individual

---

Fig. 3. Effects of 11 wk of physical exercise on the metabolic rate of glucose in segmented femoral skeletal muscle (A), vastus medialis muscle (B), vastus lateralis muscle (C), hamstring muscle (D), adductor muscle (E), and gracilis muscle (F). $MR_{glu}$, metabolic rate of glucose. Nos. of subjects: CON: Pre 14; Post 12; MOD: Pre 16, Post 15; HIGH: Pre 16, Post 15. Data are the average of bilateral means + SD. #Significant different from CON over the course of the intervention assessed by ANCOVA: $#P = 0.01–0.05$, $##P = 0.001–0.01$; *significant difference within group assessed by paired t-test: $*P = 0.01–0.05$, $**P = 0.001–0.01$, $***P = 0.0001–0.0001$, $****P < 0.0001$. 

---

The metabolic rate of glucose (MRglu) in skeletal muscle is shown in Fig. 3. The metabolic rate of glucose is significantly increased in the MOD group compared to the CON group. The increase is significant in both MOD and HIGH for the vastus medialis, vastus lateralis, and the hamstring group. The correlation between whole body insulin sensitivity at 0–60 min after FDG injection and glucose uptake of the individual muscles is shown in Fig. 3B–F. The correlation is significant for MOD, but not for HIGH.
Differential effects of exercise on regional glucose uptake

Table 1. Effects of 11 wk of physical exercise on mass and glucose metabolism of individual tissue depots

<table>
<thead>
<tr>
<th>Tissue mass</th>
<th>CON</th>
<th>MOD</th>
<th>HIGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Total body mass, kg</td>
<td>Pre 92.8 (8.5) Post 92.9 (8.5)</td>
<td>Pre 93.2 (8.1) Post 89.7 (8.6)</td>
<td>Pre 91.3 (7.2) Post 88.8 (6.8)</td>
</tr>
<tr>
<td>A Skeletal muscle, kg</td>
<td>Pre 34.5 (3.1) Post 34.6 (3.3)</td>
<td>Pre 34.2 (3.7) Post 34.5 (3.7)</td>
<td>Pre 34.5 (3.1) Post 35.2 (3.0)</td>
</tr>
<tr>
<td>A Total body fat, kg</td>
<td>Pre 29.0 (6.0) Post 28.9 (5.8)</td>
<td>Pre 30.0 (4.6) Post 25.8 (5.1)</td>
<td>Pre 27.4 (4.2) Post 23.6 (3.7)</td>
</tr>
<tr>
<td>A Leg fat free mass, kg</td>
<td>Pre 22.5 (2.3) Post 22.4 (2.3)</td>
<td>Pre 21.8 (2.8) Post 22.1 (2.6)</td>
<td>Pre 22.6 (2.5) Post 23.3 (2.6)</td>
</tr>
<tr>
<td>A Leg fat mass, kg</td>
<td>Pre 8.7 (2.4) Post 8.8 (2.4)</td>
<td>Pre 8.5 (1.9) Post 7.6 (2.0)</td>
<td>Pre 7.9 (1.3) Post 7.0 (1.3)</td>
</tr>
<tr>
<td>B Abdominal SAT, mm</td>
<td>Pre 2.9 (1.0) Post 3.0 (0.9)</td>
<td>Pre 3.0 (0.8) Post 2.6 (0.7)</td>
<td>Pre 3.0 (0.6) Post 2.5 (0.5)</td>
</tr>
<tr>
<td>B Abdominal VAT, mm</td>
<td>Pre 2.0 (0.6) Post 2.1 (0.6)</td>
<td>Pre 2.2 (0.8) Post 1.9 (0.6)</td>
<td>Pre 2.0 (0.7) Post 1.6 (0.4)</td>
</tr>
</tbody>
</table>

Km values individual tissues

| C Km skeletal muscle, 10^{-2} min^{-1} | Pre 17.6 (4.9) Post 17.6 (5.5) | Pre 18.7 (4.9) Post 22.7 (5.6) | Pre 15.7 (5.3) Post 21.3 (5.0) |
| D Km femoral SAT, 10^{-2} min^{-1} | Pre 3.8 (1.2) Post 4.3 (1.9) | Pre 4.2 (1.7) Post 4.9 (1.4) | Pre 4.0 (1.6) Post 4.7 (1.8) |
| E eKm abdominal SAT, 10^{-2} min^{-1} | Pre 7.4 (3.0) Post 6.7 (3.8) | Pre 6.3 (3.2) Post 5.3 (1.7) | Pre 5.1 (1.3) Post 5.2 (1.2) |
| F eKm abdominal VAT, 10^{-3} min^{-1} | Pre 14.2 (5.1) Post 12.3 (5.4) | Pre 11.8 (3.9) Post 11.0 (3.8) | Pre 10.4 (3.0) Post 10.9 (2.4) |

Glucose uptake per depot

| F Ru PET 0-60, mg glucose·kg (total body mass)^{-1}·min^{-1} | Pre 7.6 (2.2) Post 7.2 (1.5) | Pre 7.7 (1.9) Post 8.7 (1.7) | Pre 7.4 (2.1) Post 8.7 (2.3) |
| G Skeletal muscle, mmol/min | Pre 3,825 (1,081) Post 3,680 (744) | Pre 3,944 (915) Post 4,327 (904) | Pre 3,734 (1,024) Post 4,332 (1,251) |
| %Uptake skeletal muscle | Pre 69 (16) Post 73 (28) | Pre 71 (16) Post 81 (12) | Pre 64 (12) Post 79 (12) |
| H Femoral AT, mmol/min | Pre 178 (32) Post 179 (60) | Pre 180 (58) Post 164 (67) | Pre 161 (34) Post 153 (47) |
| %Uptake femoral AT | Pre 4 (1) Post 5 (2) | Pre 5 (2) Post 4 (1) | Pre 5 (1) Post 4 (1) |
| I Abdominal SAT, mmol/min | Pre 101 (30) Post 98 (36) | Pre 107 (58) Post 67 (22) | Pre 90 (49) Post 73 (29) |
| %Uptake abdominal SAT | Pre 2 (1) Post 3 (1) | Pre 3 (1) Post 2 (1) | Pre 2 (1) Post 2 (1) |
| J Abdominal VAT, mmol/min | Pre 122 (32) Post 121 (49) | Pre 145 (33) Post 109 (29) | Pre 122 (71) Post 88 (27) |
| %Uptake abdominal VAT | Pre 3 (1) Post 3 (1) | Pre 4 (1) Post 2 (1) | Pre 3 (1) Post 2 (0) |

Data are means (SD). CON, sedentary control group; MOD, moderate-dose exercise group; HIGH, high-dose exercise group; Pre, at baseline; Post, after intervention; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue; Km, rate constant of FDG; eKm, estimated Km; Ru PET 0-60, total body glucose disposal rate; %uptake, 100% · tissue depot uptake/Ru PET 0-60. Significant difference from CON over the course of the intervention assessed by ANCOVA: *P = 0.01–0.05, **P = 0.001–0.01; ###P = 0.0001–0.001; ####P < 0.0001; *significance difference within group assessed by paired t-test: *P = 0.01–0.05, **P = 0.001–0.01, ***P = 0.0001–0.001, ****P < 0.0001.

Adipose tissue glucose uptake. Glucose uptake as estimated by SUVmean decreased in femoral SAT in HIGH both within group (P = 0.03) and compared with CON (P = 0.02), whereas only a trend was found in MOD compared with CON (P = 0.09; Fig. 5A). Glucose uptake also decreased in anterior abdominal SAT in both physical exercise groups compared with CON (MOD P = 0.0006, HIGH P = 0.004; Fig. 5C). In the posterior abdominal SAT, SUVmean decreased only in MOD compared with CON (P = 0.01), whereas a trend toward a decrease was present in HIGH (P = 0.08; Fig. 5E). No change was found in SUVmean for either intra- or retroperitoneal VAT (P > 0.1; Fig. 6, A and C).

When calculated as MRglu, no difference was observed in femoral SAT either within or between groups (P > 0.1; Fig. 5B).

MRglu was estimated for abdominal SAT and VAT VOIs, as described in MATERIALS AND METHODS, but for this analysis the numbers of subjects were only 8, 10, and 12 in CON, MOD, and HIGH, respectively. SUVmean and eMRglu correlated at muscles ranged from r = 0.49 (gracilis) to r = 0.68 (hamstrings) (all P < 0.0001). At baseline, MRglu in gracilis was lower than in all other muscle groups (P < 0.0001). Also, MRglu in vastus medialis was higher than in the hamstrings (P = 0.02), but no other significant differences were found in MRglu between any of the examined skeletal muscle regions when compared pairwise. The glucose uptake of individual muscle or muscle groups were pairwise associated with r values ranging from 0.67 (P < 0.0001) for vastus medialis and the hamstrings to 0.97 (P < 0.0001) for vastus medialis and vastus lateralis.

Skeletal muscle GLUT4. The transport capacity for glucose across the muscle fiber membrane was assessed as GLUT4 protein expression. A within-group increase of GLUT4 expression was found for both exercise groups in both the basal (MOD P = 0.006, HIGH P = 0.011; Fig. 4A) and the insulin-stimulated state (MOD P = 0.04, HIGH P = 0.003; Fig. 4B). Compared with CON, in the basal state the difference was still significant for MOD (P = 0.05), whereas only a trend remained in HIGH (P = 0.08). No correlation was found between GLUT4 expression and MRglu of vastus lateralis or segmented skeletal muscle either at baseline or for intervention-induced changes (P > 0.1).
baseline for anterior (r = 0.78, P < 0.0001) and posterior abdominal SAT (r = 0.81, P < 0.0001) as well as for intra- (r = 0.76, P < 0.0001) and retroperitoneal VAT (r = 0.72, P < 0.0001). As for SUs mean (Fig. 5C), eMR glu (Fig. SD) decreased within group in anterior abdominal SAT and in MOD (P = 0.04). Likewise, a trend toward a within-group decrease was observed for eMR glu in MOD in posterior abdominal SAT (Fig. 5F; P = 0.07) and intraperitoneal VAT (Fig. 6B; P = 0.095). No change in eMR glu was found within group in retroperitoneal VAT of MOD or HIGH (P > 0.1; Fig. 6D).

At baseline, insulin-stimulated glucose uptake (both SUV mean and eMR glu) was higher in intra- than in retroperitoneal VAT (P < 0.001; Fig. 6). Glucose uptake in both intra- and retroperitoneal VAT was higher than glucose uptake in both the anterior and the posterior abdominal SAT (P < 0.0001; Figs. 5 and 6). No difference was found between glucose uptake in anterior vs. posterior abdominal SAT (P > 0.1), whereas both were higher than glucose uptake in femoral SAT (P < 0.0001; Fig. 5). At baseline, whole body glucose uptake at 0–60 min after FDG injection correlated positively with eMR glu in anterior and posterior abdominal SAT and in intra- and retroperitoneal VAT (eMR glu: r = 0.45–0.54, P < 0.01).

Adipose tissue masses and leg FFM. Abdominal SAT, VAT, and leg fat masses decreased similarly in the two intervention groups compared with CON (Table 1). Leg FFM increased in HIGH compared with CON (P = 0.0007), and the increase in HIGH tended to be higher than in MOD (P = 0.06). No difference was found in MOD compared with CON (P > 0.1; Table 1).

At baseline, inverse correlations were found between VAT mass and SUV mean for both intra- (r = −0.38, P = 0.04) and retroperitoneal VAT (r = −0.41, P = 0.03). No correlation was found between VAT mass and skeletal muscle MR glu (P > 0.1) or whole body glucose uptake at 0–60 min after FDG injection (P > 0.1) or between VAT or SAT mass and glucose uptake in SAT (P > 0.1).

Total glucose uptake rate per tissue depot. Total body glucose R d (µmol/min) increased within group in HIGH (P = 0.03) with a remaining trend compared with CON (P = 0.07), whereas no significant difference was found in MOD (P > 0.1; Table 1). Total skeletal muscle glucose uptake rate increased within group in both MOD (P = 0.007) and HIGH (P = 0.002) (Table 1). Compared with CON, the increase was still significant in MOD (P = 0.02), whereas only a trend was found in HIGH (P = 0.06). No change was observed in the total glucose uptake rate of femoral adipose tissue for either intervention group (P > 0.1; Table 1). Abdominal SAT glucose uptake rate decreased within group in MOD (P = 0.04) and tended to decrease compared with CON (P = 0.05), whereas no change was found for HIGH (P > 0.1; Table 1). For the abdominal VAT, a within-group decrease was found in MOD (P = 0.004; Table 1). In VAT in HIGH, a tendency toward a decrease was found within group (P = 0.09), which remained compared with CON (P = 0.08).

DISCUSSION

This is the first randomized controlled trial directly examining effects of endurance training on insulin-stimulated glucose uptake in individual regions of skeletal muscle and adipose tissue by use of FDG PET/CT methodology. A main finding was that moderate- and high-dose physical exercise increased the insulin-stimulated glucose uptake rate similarly for all femoral skeletal muscle groups investigated. GLUT4 expression in skeletal muscle (vastus lateralis) also increased similarly in both exercise groups. Another main finding was that physical exercise affected glucose uptake differentially in adipose tissue and skeletal muscle. Endurance training did not change the insulin-stimulated glucose uptake rate in femoral SAT or intra- or retroperitoneal VAT, whereas the insulin-stimulated glucose uptake, as estimated by SUV mean, decreased after physical exercise in both anterior and posterior abdominal SAT. Moreover, mass of abdominal VAT and SAT and leg fat mass decreased to the same extent with moderate- and high-dose physical exercise.

The PET/CT scans were performed during a hyperinsulinemic-isoglycemic clamp, enabling us to ensure a steady blood glucose level and to calculate the peripheral insulin sensitivity (30). A correlation of r = 0.63 was observed between steady-state whole body insulin sensitivity measured by the clamp technique at 0–60 min after FDG injection and PET segmented femoral skeletal muscle glucose uptake. The correlation was a little weaker than previously reported in other FDG-PET studies (r = 0.75–0.85) (14, 22, 39), which might be ascribed to our narrowly selected subject population. At baseline, the skeletal muscle glucose uptake rate of the present...
skeletal muscle glucose uptake. This experimental situation is not the rate-limiting step for glucose uptake rate. This indicates that GLUT4 expression for expression at baseline associated with baseline skeletal muscle increases of any of these three parameters; nor was the GLUT4 no correlations were found between the intervention-induced the increase in skeletal muscle GLUT4 expression. However, determined by the clamp technique at 90 –120 min as well as PET study (39). The increase in skeletal muscle glucose uptake rate found in endurance-trained men in a cross-sectional FDG uptake rate paralleled the increase in whole body glucose uptake (16, 44). SUVmean might have underestimated glucose uptake (16, 44). SUVmean might have underestimated glucose uptake calculated as standardized uptake value and metabolic rate of glucose in femoral adipose tissue (A and B), anterior abdominal subcutaneous adipose tissue (C and D), posterior abdominal subcutaneous adipose tissue (E and F). Data assessed by FDG PET/CT and automatically segmented into individual tissue regions. SUV, standardized uptake value; $M_{\text{R}_{\text{glu}}}$, metabolic rate of glucose; e, estimated. Nos. of subjects: (A and B) CON: Pre 13, Post 12; MOD: Pre 15, Post 14; HIGH: Pre 14, Post 13; (C and E): CON: Pre 15, Post 12; MOD: Pre 15, Post 16; HIGH: 16. (D and F): CON: Pre 9, Post 8; MOD: Pre 11, Post 10; HIGH: 12. Data are average of individually bilateral means + SD. #Significant difference from CON over the course of the intervention assessed by ANCOVA; $\#P = 0.01–0.05$, $\##P = 0.001–0.01$, $\###P = 0.0001–0.001$; *significant difference within group assessed by a paired t-test; $^*P = 0.01–0.05$.

Exercise significantly increased femoral skeletal muscle glucose uptake in general as well as in the individual femoral muscle groups, and the observed postintervention glucose uptake rate of the exercise groups corresponded to the uptake rate found in endurance-trained men in a cross-sectional FDG PET study (39). The increase in skeletal muscle glucose uptake rate paralleled the increase in whole body glucose uptake determined by the clamp technique at 90 –120 min as well as the increase in skeletal muscle GLUT4 expression. However, no correlations were found between the intervention-induced increases of any of these three parameters; nor was the GLUT4 expression at baseline associated with baseline skeletal muscle glucose uptake rate. This indicates that GLUT4 expression for this experimental situation is not the rate-limiting step for skeletal muscle glucose uptake.

We chose to acquire dynamic PET scans of the femoral region, whereas the PET scans of the abdominal region were static, enabling direct quantification of the glucose uptake rate in the abdominal region. To estimate glucose uptake in abdominal adipose tissue, we used the surrogate measure of SUVmean. Although SUVmean is indeed usable in cancer descriptive diagnostics, it is a semiquantitative parameter with respect to glucose uptake and has several methodological reservations, as many assumptions must be met in order for SUVmean to reflect glucose uptake (16, 44). SUVmean might have underestimated adipose tissue glucose uptake after the exercise interventions due to the training-induced increase in skeletal muscle glucose uptake and consequently lower plasma FDG. To validate our findings, we applied a simplified method to estimate the insulin-stimulated metabolic rate of glucose ($eM_{R_{\text{glu}}}$) in abdominal adipose tissue, in which we used whole blood FDG to estimate $K_m$. However, as we did not have the necessary blood samples from all subjects, the number of subjects for calculations of $eM_{R_{\text{glu}}}$ were lower than for calculations of SUVmean, resulting in a large data variation. This increased the risk of a type II error, but in general, conclusions drawn based on $eM_{R_{\text{glu}}}$ and SUVmean support each other. A further limitation of the study was that we did not have sufficient power to detect or
defer small differences in the response to exercise training between the MOD and HIGH training groups or to differentiate the response to exercise between the individual muscle groups.

In accord with other studies (3, 20, 41, 42) we observed a higher glucose uptake in abdominal VAT than in SAT, which might have been due not only to an increased cellularity of VAT compared with SAT but also to a greater glucose uptake in each adipocyte and/or stroma-vascular cell in VAT (3). We also found higher uptake of glucose in intraperitoneal than in retroperitoneal VAT. While this has not previously been examined in humans, no difference was observed between insulin-stimulated glucose uptake in mesenteric, parametrial, and abdominal SAT by microdialysis technique before and after 12 wk of physical exercise (6 days/wk, 45 min/day at 70% of \( V\overline{O}_{2\text{max}} \)) in rats (retroperitoneal, parametrial, and mesenteric VAT and SAT) (9).

Despite the exercise-induced increase in whole body insulin sensitivity and skeletal muscle glucose uptake, we found no increase in insulin-stimulated glucose uptake in abdominal VAT or in femoral or abdominal SAT after 11 wk of physical exercise. In accord with this, weight loss after a 6-wk very-low-calorie diet resulted in no changes in perfusion or glucose uptake (per kg tissue) in either abdominal VAT or SAT as determined by PET (41). Dela and Stallknecht (7) determined the insulin-stimulated glucose uptake in abdominal and femoral SAT by microdialysis technique before and after 12 wk of physical exercise (6 days/wk, 45 min/day at 70% of \( V\overline{O}_{2\text{max}} \)) in first-degree relatives of type 2 diabetics and control subjects and likewise found no effect of physical exercise. However, long-term and/or very high amounts of exercise may increase the insulin-stimulated glucose uptake in adipose tissue as determined by comparing athletes and sedentary lean men (abdominal SAT) (37) as well as 15 wk of swim training (6 h/day, 5 days/wk) in rats (retroperitoneal, parametrial, and mesenteric VAT and SAT) (9). Interestingly, Højbjerg et al. (15) found an increase in insulin-stimulated abdominal SAT glucose uptake as measured by microdialysis after 10 days of bed rest in lean, healthy subjects and at the same time a decrease in peripheral insulin sensitivity as determined by a hyperinsulinemic, isoglycemic clamp. Moreover, we found, in two previous studies by use of microdialysis technique, a higher insulin-stimulated glucose uptake in abdominal SAT of whole body insulin-resistant relatives of type 2 diabetics compared with insulin-sensitive control subjects (7, 15). In the present study, endurance training of overweight men increased peripheral and skeletal muscle insulin sensitivity, but the insulin-stimulated glucose uptake in adipose tissue did not change or decreased, depending on the type of adipose tissue and prerequisites in calculations. These findings indicate no association or maybe even an inverse association between physical activity-induced changes in insulin-stimulated glucose uptake in skeletal muscle and adipose tissue in humans. The mechanism could include secretion of myokines by insulin-sensitive skeletal muscle signaling to adipose tissue that glucose uptake should be decreased in order to maintain glucose homeostasis (27). In addition, physical exercise has been shown to increase insulin-stimulated glucose uptake only in contracting muscles and not in those muscles that are not active during exercise training (6, 11, 40). Extrapolating this to adipose tissue (which does not contract), an exercise-induced increase in insulin-stimulated glucose uptake might not be expected in adipose tissue. Finally, it is possible that the ability of insulin to stimulate glucose uptake in adipose tissue requires the presence of glucose-dependent insulinoactive polypeptide (GIP) or another incretin (2), as would be the case after intake of nutrients per os.

We observed a decrease of VAT, abdominal SAT mass, and leg fat mass, which was similar in the two exercise groups and an inverse association at baseline between VAT mass and glucose uptake in both intra- and retroperitoneal VAT. This inverse association is in accord with cross-sectional findings by the Turku
group, where obese type 2 diabetic patients and healthy obese and lean controls were examined with FDG PET during a hyperinsulinemic-euglycemic clamp (42). In the longitudinal part of the present study, despite loss of adipose tissue mass, physical exercise did not increase glucose uptake of adipose tissue either per kilogram of tissue or per tissue depot. If the cell mass is reduced by decreasing lipid content alone and the FDG uptake per cell is constant, then the recorded glucose uptake per unit mass in the adipose tissue (and therefore SUV and eMRglu) would increase, as there are more cells/cytoplasm per voxel or kilogram of tissue. In our study, the adipose tissue mass of the individual depots decreased, and SUV and eMRglu of the individual tissues decreased or remained unchanged. Therefore, we have found indications of a decrease in adipocyte glucose uptake after training.

The PET FDG method has inherent limitations. PET scans have limited resolution, and in a border region between two tissues, the measured glucose uptake values are influenced by both tissues (partial volume effects). To minimize the effect, which in particular might influence the low glucose uptake values in femoral SAT, all segmented ROIs were shrunk by three pixels prior to the kinetic analysis. This, however, limits statistics of the measurement. Due to the low-dose CT, segmentation method, and subsequent shrinking of ROIs, use of MRI was necessary to assess the mass of abdominal VAT and SAT, while DEXA was used for the estimation of leg fat mass. Furthermore, we used an IDIF based on the femoral artery as a surrogate measurement of the arterial blood FDG concentration. However, we calibrated this measurement against arterial FDG values in a subpopulation. Previous studies have used a simplified model (Patlak analysis) for the estimation of glucose metabolism (25, 26). In our study, values from adipose tissues were found not to be well described by the simple model, and a full kinetic model calculation was performed on the dynamic scan data. This showed a significant k4 (leak out of tracer) in the adipose tissues, not described by others. Tissue heterogeneity may cause a similar effect (33, 34). However, spatial heterogeneity is well known within skeletal muscle (29), and we did not observe any substantial k4 in this tissue.

In conclusion, 11 wk of daily moderate- and high-dose aerobic exercise similarly increased the insulin-stimulated glucose uptake in skeletal muscle (per kg muscle and for the total depot) both in femoral muscle in general and in five individual femoral muscle groups. However, no effect of physical exercise was observed on insulin-stimulated glucose uptake in intra- or retroperitoneal VAT or in femoral SAT. Insulin-stimulated glucose uptake in abdominal SAT seemed to decrease. These findings indicate that exercise training favors increased insulin-stimulated glucose uptake in skeletal muscle in contrast to adipose tissue, which might be of importance for maintenance of glucose homeostasis in exercise trained individuals.

ACKNOWLEDGMENTS

We acknowledge and thank the subjects who participated in the study as well as all students and technical staff that made this intervention work: Astrid Permile Jespersen, Julie Bønnelycke, Thomas Christian Bonne, Line Quist Bendtsen, Charlotte Stephansen, Marie-Louise Udenggaard, Fima Sigurðardottir, Sine Winther Nielsen, Helle Roager Jensen, Jonas Salling Kjeldsen, Mia Lundby Krågelund, Anne SofieGran, Jannie Østergaard, Hanne Thorvig, Julie Agerschou, Thomas Beck, Gerda Hau, Jakob Utzon-Frank, Karin Stahr, Marianne Federspiel, Camilla Sloth Knudsen, Elizabeth Abrahamson, Johanna Kronvall, Adam Espe Hansen and Kristian Roebbe. We thank Novo Nordisk A/S for the donation of the mouse monoclonal antibody used for Western blotting to determine GLUT4 expression. We also thank Prof. Jørgen F. Wojtaszewski and Erik A. Richter for donation of the buffer used for homogenization of muscle biopsies and Assoc. Prof. Lene Theil Skovgaard for helpful discussions concerning the statistical procedures.

GRANTS

This work was carried out as a part of the research program of the UNIK: Food, Fitness & Pharma for Health and Disease (see http://foodfitnesspharma.ku.dk). The UNIK project is supported by the Danish Ministry of Science, Technology and Innovation. Further financial support was obtained from the Novo Nordisk Foundation and Center for Healthy Aging. The PET/CT scanners were donated by the John and Birthe Meyer Foundation.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


