Muscle-specific glucose and free fatty acid uptake after sprint interval and moderate-intensity training in healthy middle-aged men

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PREVALENCE AND INCIDENCE of metabolic diseases, especially obesity and type 2 diabetes, are increasing at alarming rates. This is strongly associated with a current sedentary lifestyle and lack of appropriate physical activity and exercise. Research over the past decades has clearly shown the beneficial effects of regular physical activity and exercise on fitness and many health-related parameters pertaining to the function of the human body. However, which exercise training mode provides the best health benefits and would be optimal for exercise recommendations to lifestyles of people in current Western societies remains unknown.

An emerging amount of studies shows that not only continuous, longer-duration moderate-intensity training (MIT), but also strenuous sprint interval training (SIT) produces health-enhancing changes in the body with much less time-commitment. Among others, these include improved maximum O2 uptake (11), skeletal muscle oxidative capacity (3, 4), whole body fat oxidation (3, 38), and peripheral vascular function (27) by SIT. Very striking are the effects of SIT on whole body glucose metabolism, as evidenced by markedly decreased glucose and insulin responses demonstrated in oral glucose tolerance test (OGTT) (1). However, large effects have not been confirmed in all studies employing different high-intensity interval training programs (31, 37, 38). The training-induced improved insulin-mediated glucose uptake (GU) rates at the whole body level may be due to an effect in many tissues, such as in the liver or adipose tissue, but it is mostly attributed to an effect on skeletal muscle (8, 9, 28). Favoring also the latter are the findings of a large increase in GLUT-4 content in the vastus lateralis (VL) muscle by short-term SIT (2, 22). These findings indirectly suggest that GU in that particular muscle could also be increased. On the other hand, whether the responses are similar in the three other quadriceps muscles and between the leg and arm muscles when the subjects are using certain leg muscles, mainly quadriceps femoris (QF), activating cycle ergometer exercise remains currently unknown. It has been postulated that one of the mechanisms why SIT particularly in cycling is so effective is that intense cycling exercise activates different muscle fiber types and muscles also in the upper body more comprehensively than moderate-intensity exercise, thereby providing basis for metabolic adaptation throughout all the muscles of the body (1).

While OGTT and euglycemic hyperinsulinemic clamp measurements provide important information on whole body glucose metabolism and insulin sensitivity, positron emission tomography (PET) imaging technique has the unique potential to reveal tissue- and region-specific differences in energy substrate metabolism and insulin sensitivity. In the present study, we used PET combined with fluorine-18 labeled tracers, 2-deoxy-2-(18F)fluoro-D-glucose (18F-FDG) and 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid (18F-FTHA), to measure glucose and free fatty acid uptake (FFAU) in relevant muscles of lower and upper body in response to SIT and MIT training performed by cycling. Our first aim was to investigate the regional differences in improvements of muscle insulin-stimulated GU between short-term SIT and MIT interventions. Our hypothesis was that SIT would induce a larger increase in GU in the upper body muscles and a more uniform increase between the individual QF muscles than MIT. Our second aim was to investigate the effects of SIT and MIT on FFAU in different muscles. These latter measurements were performed in the non-insulin-stimulated state. The hypothesis was that the...
FFAU would also be increased due to training and similarly in a muscle-specific manner between the training modes as GU.

METHODS

Subjects

Twenty-eight sedentary, nonsmoking, middle-aged (mean 48 yr, range 40–55 yr), healthy men were recruited into this study with newspaper advertisements, through personal contacts, and by using electronic and traditional bulletin boards, as previously described (20). They were nonobese, did not do exercise on a regular basis (twice a week or less), had no previous active training background, and had peak oxygen consumption ($V\text{O}_2^{peak} < 40 \text{ ml kg}^{-1} \text{ min}^{-1}$) to complement the information about training status (20). The health status of the subjects was determined by a thorough physical examination to determine any condition that, in the opinion of the investigator, could create a hazard to the participant’s safety, endanger the study procedures, or interfere with the interpretation of the study results. The purpose, nature, and potential risks were verbally and literally explained to the subjects before they gave their informed consent to participate. The study was performed according to the Declaration of Helsinki and was approved by the Ethical Committee of the Hospital District of South-Western Finland (decision 95/180/2010 §228). The present study is a part of a larger study entitled, “The Effects of Short-Term High-Intensity Interval Training on Tissue Glucose and Fat Metabolism in Healthy Subjects and in Patients with Type 2 Diabetes” (NCT01344928).

Study Design

Pretraining OGTT was performed during the screening day to ensure that the subjects did not have impaired glucose tolerance to begin with. This was followed by a $V\text{O}_2^{peak}$ test (20) on the same evening or another day. Both OGTT and $V\text{O}_2^{peak}$ test are described in more details below in the text. At least 1 wk later from the screening day, an $18F$-FDG-PET study was performed to measure FFAU in different muscles (see details below). The following morning, euglycemic hyperinsulinemic clamp was conducted, starting with the tracer injection at 90 min (SD 16) after the start of the clamp. Euglycemic hyperinsulinemic clamp was continued until the end of the PET study. Subjects were required to have fasted for at least 10 h before the OGTT and PET measurement days. They were also instructed to abstain from caffeinated drinks and to avoid exhausting exercise 48 h before the studies. After all pretraining measurements, the subjects were randomly divided into two training groups: into either the SIT or MIT groups.

After training intervention of 2 wk, all of the measurements were repeated, starting on the second day (~48 h) after the last training session. On the 1st day, an $18F$-FTHA-PET study was performed. On the next day, euglycemic hyperinsulinemic clamp and $18F$-FDG-PET study during the clamp were performed. Finally, on the 4th posttraining measurement day, OGTT and $V\text{O}_2^{peak}$ test were performed. During the intervention, one participant from the SIT group dropped out due to training-induced hip pain and one from the MIT group due to personal reasons.

Training Interventions

As previously described (20), both groups (SIT and MIT) trained six sessions within 2 wk in controlled laboratory conditions. The progressive SIT training included 4–6 × 30 s of all-out cycling efforts with 4 min of recovery during which participants were allowed to remain still or do unloaded cycling (Monark Ergomedic 828E; Monark, Vansbro, Sweden), based on the protocol originally described by Burgomaster et al. (4). The participants were quickly familiarized with the SIT training protocol (~1 wk before the intervention). The initial number of bouts was four, and it was increased by one after every other session. Each bout started with 5-s acceleration to maximal cadence without any resistance, followed by a sudden increase of the load (7.5% of whole body weight in kg) and maximal cycling for 30 s (Wingate protocol). A session of MIT group consisted of 40–60 min of cycling at an intensity of 60% of $V\text{O}_2^{peak}$ intensity with electrically braked ergometer (Tunturi E85, Tunturi Fitness, Almere, the Netherlands). The duration of cycling was initially 40 min, and it was increased by 10 min after every other session until 60 min was reached during the last two sessions.

We calculated energy consumption during the training over all six training sessions. Calculation was done, individually, based on the average intensity over the sprints, time spent on sprints (15 min in total), and estimated efficiency of 20% in cycling for the SIT group. Time spent on pauses between the sprints and warm-up and cool down were not included in the calculation. For the MIT groups, we used correspondingly the average intensity (60% of $V\text{O}_2^{peak}$ intensity), time spent on training (300 min in total), and estimated efficiency of 20% in cycling. Also, for the MIT, warm-up and cool down were not included in the calculation.

PET Measurements

Before the PET experiments, antecubital veins from the both arms were cannulated. One catheter was for the administration of PET tracers and for the other infusions (glucose-insulin clamp) and the opposite for blood sampling. The arm that was used for blood sampling was heated with an electrically powered cushion to arteri- lize the venous blood for the length of the study. The subject was positioned into the PET scanner in a supine position with the femoral region and for the upper body muscle measurements with the thoracic region in the scanning area of the gantry. The PET and computed tomography (CT) imaging was performed with GE Advance PET/CT scanner (General Electric Medical System, Milwaukee, WI).

FFAU was measured using $18F$-FTHA that was produced as previously described (7, 23). The tracer [155 MBq (SD 9)] was injected into the vein, and scanning of the thoracic region (biceps and triceps brachii muscles) was started simultaneously and continued for 40 min in $4 × 15\text{ s}$, $6 × 20\text{ s}$, $2 × 60\text{ s}$, $2 × 150\text{ s}$, and $6 × 300\text{ s}$ time frames. Starting at 4 min after the tracer injection, blood samples for plasma radioactivity determination (Wizard 1480 3; Wallac, Turku, Finland) and calculation of input function were collected at approximately (exact timing was recorded) 4-, 5-, 7.5-, 10-, 20-, 30-, and 40-min time points. As the heart was in the imaging area, the activity in the left ventricular chamber in the first 10 frames (first 5 min) in PET image set were used for determination of input function for that period. The femoral region (QF and hamstring muscle groups) was scanned starting at ~65 min after the tracer injection in $3 × 300\text{ s}$ time frames. Finally, the shoulder region was scanned starting at ~90 min after the tracer injection in $3 × 300\text{ s}$ time frames. In the middle of each frame during both femoral and shoulder region scans, a blood sample for plasma radioactivity determination was taken and used for the input function calculation. Other blood samples were collected at ~5, 10, 20, 30, 40, and 50 min after the tracer injection to measure the metabolites of the $18F$-FTHA in the blood and to later correct them from the input function to obtain pure plasma $18F$-FTHA input function.

GU was measured using $18F$-FDG that was produced as previously described (14, 21). The tracer [157 MBq (SD 10)] was injected into the vein, and similar scans with similar framing from thoracic, femoral, and shoulder regions were performed as in the $18F$-FTHA-study. Also plasma radioactivity samples for input function were taken similarly, but no other samples were needed as $18F$-FDG is not metabolized.
PET Data Analysis

All PET image data were corrected for dead time, decay, and photon attenuation and reconstructed using 3D-OSEM reconstruction. Carimas software (version 2.7, Turku PET Centre, Turku, Finland, www.turkupetcentre.fi/carimas) was used in the image analysis. Regions of interest (ROIs) were drawn into the CT images manually and copied into the PET images for the data analysis. Femoral region ROIs encompassed the cross section of the whole QF, the four heads of QF individually [rectus femoris (RF), VL, vastus intermedius (VI), and vastus medialis (VM)], and the hamstring (semimembranosus, semitendinosus, and biceps femoris) in five subsequent cross-sectional mid thigh planes (5×3.3 mm thick). For the upper body muscles, ROIs were drawn into the deltoids and biceps and brachii muscles also on five subsequent cross-sectional mid muscle planes. Using these ROIs, tissue activity curves were extracted from the PET data.

Fractional rate of tracer uptake was calculated using graphical analysis (25) from tissue activity curves and input function. The rate of GU and FFAU was calculated by multiplying the fractional tracer uptake rate by the plasma glucose or plasma free fatty acid (FFA) concentration of the study subject during the PET scan. With GU, this was further divided by the lumped constant value of 1.2 (26). Analyses of plasma FFA and glucose concentrations were performed at the Turku University Hospital using standard assays.

Other Measurements

OGTT and euglycemic hyperinsulinemic clamp study. A 2-h OGTT was done after the subjects had fasted at least for 10 h. To start the test, the subject drank a 330-ml solution containing 75 g of glucose (Nutrical, Nutricia Medical, Turku, Finland). Blood samples were collected before drinking and at 15, 30, 60, 90, and 120 min after drinking and were used to determine the glucose and insulin concentrations in the blood. Area under curve of the glucose and insulin responses during the whole 2-h period was calculated by assuming linearity of the curve between the sample times using Microsoft Excel (Microsoft, Redmond, WA).

Euglycemic hyperinsulinemic clamp was based on the original description by Defronzo and colleagues (6). In the present study, clamp was performed after the subjects had fasted at least for 10 h. First the antecubital veins of the both arms were cannulated. A primed-constant insulin (Actrapid, 100 U/ml, Novo Nordisk, Bagsvaerd, Denmark) infusion was started with the rate of 40 μU·m⁻²·min⁻¹ of body surface area in minutes during the first 4 min. After 4 min and up to 7 min, infusion rate was reduced to 20 μU·m⁻²·min⁻¹ and, after 7 min to the end of the clamp, it was kept constant at 10 μU·m⁻²·min⁻¹. Exogenous glucose infusion was started 4 min after the start of the insulin infusion with a rate of subject’s weight (kg)0.1·1·g⁻¹·h⁻¹. At 10 min, glucose infusion was doubled, and after that further adjusted according to blood glucose concentration to keep it as closely as possible to the level of 5 mmol/l. Arterialized venous blood samples were collected before the clamp and every 5 min during the first 30 min of the clamp to determine the glucose concentration for adjusting the glucose infusion rate. After 30 min, samples were taken every 10 min to check the glucose level and to make adjustment in glucose infusion rate if needed. Whole body insulin-stimulated GU rate was calculated from the measured glucose values collected during the PET scan that was started 90 min (SD 16) after the start of the clamp. FDG-PET study was performed when the subject had reached the stable glucose concentrations at the level of 5 mmol/l (within ±5% range for at least 15 min) after positioning into the PET scanner.

V̇O₂ peak test and body composition. As previously described (20), the subjects performed an exercise test (V̇O₂ peak test) on a bicycle ergometer (Ergoline 800s; VIASYS Healthcare, Germany) to determine V̇O₂ peak. The participants were asked to abstain from eating and drinking at least for 2 h before the testing. The test was started at 50 W and followed by an increase of 30 W every 2 min until volitional exhaustion. Ventilation and gas exchange (Jaeger Oxycon Pro; VIA-SYS Healthcare) were measured and reported as the mean value per minute. The peak respiratory exchange ratio was at least 1.15, and the peak blood lactate concentration, measured from capillary samples obtained immediately and 1 min after exhaustion (analyzed using YSI 2300 Stat Plus; YSI Incorporated Life Sciences, Yellow Springs, OH), was at least 8.0 mmol/l for all of the tests. The highest 1-min mean value of oxygen consumption was used as V̇O₂ peak. The test was performed for each participant before the intervention and 4 days after the last training session at approximately the same time of day at the test laboratory of the Paavo Nurmi Centre, University of Turku, Turku, Finland. Body composition was measured also at the Paavo Nurmi Centre using a bioimpedance monitor (InBody 720, Mega Electronics, Kuopio, Finland).

Statistical Analysis

All demographic data are presented as mean (95% confidence interval). Normal distribution of the variables was evaluated visually and tested using Shapiro-Wilk test. Logarithmic transformations were done to achieve the normal distribution assumption for all GU and FFAU data, as well as for insulin concentrations during the FTHA study.

The difference in age and height between the groups was tested using t-test. For all other parameters reported in Tables 1 and 2, we used a hierarchical linear mixed model, including one within-factor (time) and one between-factor (group). Unstructured covariance structure was used for time. Subjects with missing values (drop outs, technical problems) are included in this model, and model-based mean (SAS least square means) values are reported from all of the parameters.

For the PET data, comparisons between time (pre- vs. postmeasurements), groups, and muscle regions were performed with a hierarchical linear mixed model, including two within-factors (time and muscle region) and one between-factor (group). Unstructured covariance structure was used for time and compound symmetry covariance structure for muscle region. Subjects with missing values (drop outs, technical problems) are included in this model. We report model-based mean (SAS least squares means) values from all of the parameters measured before and after the training.

All tests were performed as two-sided with a significance level set at 0.05. The analyses were performed using SAS System, version 9.3 for Windows (SAS Institute, Cary, NC).

RESULTS

Subjects and Whole Body Findings

See Table 1. Body weight was not significantly altered by training. In the whole study population, whole body fat percentage decreased, and fat free mass increased statistically significantly. However, the mean changes were similar in both training modes. Whole body insulin sensitivity (M-value in glucose-insulin-clamp) and V̇O₂ peak increased significantly by SIT (mean increases 12% and 6%) and MIT (mean increases 8% and 3%) without a significantly different response between the groups. Glucose and insulin areas under the curve in OGTT were not different between the groups and did not change significantly by training. Calculated energy consumption during the training over the all training sessions was 403 (365, 442) kcal for the SIT group and 2,680 (2,474, 2,886) kcal for the MIT group (P < 0.001 between the groups).
Concentration of FTHA, 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid; FDG, 2-deoxy-2-(18F)fluoro-D-glucose. FTHA study was measured in the fasting state. There is no mean change between Pre and Post. Training effect indicates whether mean changes are different between study groups. SIT, sprint interval training; MIT, moderate-intensity training; BMI, body mass index.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SIT</th>
<th>MIT</th>
<th>Training Group</th>
<th>Training × Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>14</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>47 (45, 50)</td>
<td>48 (45, 51)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Height, cm</td>
<td>180 (177, 182)</td>
<td>179 (176, 181)</td>
<td>0.53</td>
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<td>Weight, kg</td>
<td>83.1 (78.2, 88)</td>
<td>82.6 (77.7, 87.4)</td>
<td>0.53</td>
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<td>BMI</td>
<td>25.9 (24.5, 27.2)</td>
<td>25.7 (24.3, 27)</td>
<td>0.76</td>
<td></td>
</tr>
<tr>
<td>Fat, %</td>
<td>20.9 (19.1, 22.8)</td>
<td>20.1 (18.3, 22)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>Total body fat free mass, kg</td>
<td>64.4 (61.7, 67.2)</td>
<td>64.8 (62.7, 67)</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>VO2peak, ml·kg⁻¹·min⁻¹</td>
<td>34.7 (32.4, 37.1)</td>
<td>36.7 (34.1, 39.3)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>M-value, μmol·kg⁻¹·min⁻¹</td>
<td>38.1 (29.7, 46.6)</td>
<td>42.7 (33.7, 51.7)</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Glucose AUC in OGTT</td>
<td>824 (746, 902)</td>
<td>869 (783, 955)</td>
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<td>3,842 (3,934)</td>
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</table>

Age and height values are means [95% confidence interval (CI)]; all other values are model-based means [95% CI]; n, no. of subjects. Group P value indicates level differences over the study. Training effect indicates whether there is mean change between Pre (Pre) and postmeasurements (Post). Training × group describes whether mean changes are different between study groups. SIT, sprint interval training; MIT, moderate-intensity training; BMI, body mass index. VO2peak, Peak oxygen consumption during the exercise test. M-value, whole-body glucose consumption during glucose-insulin clamp, AUC, area under curve, OGTT, oral glucose tolerance test. Significant differences are in bold.

Glucose, FFA, and Insulin Concentrations during the PET Studies

See Table 2. Glucose, FFA, and insulin concentrations were measured in the fasting state during FFAU measurements and under euglycemic hyperinsulinemic clamp during GU measurements. Glucose, FFA, and insulin concentrations during GU measurements (under glucose-insulin clamp) were similar between the groups and were not affected by training. In addition, glucose and insulin concentrations during FFAU measurements (fasting state) were not different between the groups and were not affected by training. The only parameter that was affected by training was FFA concentration during FFAU measurements (fasting state). It decreased by training but was not statistically differently between the groups.

GU in Different Lower and Upper Body Muscles

Examples of GU PET images in one subject from the SIT group and one subject from the MIT group before and after training interventions are shown in Fig. 1. GU was measured both in thigh muscles (QF and hamstrings) and in upper body muscles (deltoids, biceps, and triceps brachii). The training response was not significantly different between the groups (P = 0.14 for the group × muscle × training interaction), but differed between the muscles (P < 0.001 for muscle × training interaction) (Fig. 2, A and B). The training response was significant only in QF, and it was also significantly larger compared with the other four muscles (P < 0.001). In the SIT group, QF GU increased significantly (P = 0.002) by 53% and in MIT group by 28%. However, despite these differences in mean changes, the training response was not statistically significantly different between the groups due to large individual variation in the training responses.

We also investigated the responses in the four heads of QF (Fig. 3, A and B). GU increased statistically similarly with both training modes in the vasti muscles (VL, VI, and VM) (P > 0.4 in all comparisons between vasti muscles). Interestingly, the training response differed significantly (P < 0.013 in RF compared with all vasti muscles) between SIT and MIT in RF in which SIT increased GU similarly as in other three muscles, but MIT had no effect.

FFAU in Different Lower and Upper Body Muscles

In the analysis of the five larger muscles (Fig. 2, C and D), FFAU was similar between the groups (P = 0.73) and the variation in the training responses.

Table 2. Plasma glucose, FFA, and insulin concentration in the SIT and MIT study groups before and after the training interventions of 2 wk

<table>
<thead>
<tr>
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<tr>
<td>Glucose (FTHA study), mmol/l</td>
<td>5.4 (5.1, 5.7)</td>
<td>5.2 (4.9, 5.4)</td>
<td>0.20</td>
<td>0.054</td>
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<td>Glucose (FDG study), mmol/l</td>
<td>4.9 (4.7, 5.2)</td>
<td>4.8 (4.6, 5.1)</td>
<td>0.94</td>
<td>0.25</td>
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<td>FFA (FTHA study), mmol/l</td>
<td>0.61 (0.51, 0.71)</td>
<td>0.59 (0.51, 0.67)</td>
<td>0.04</td>
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<td>FFA (FDG study), mmol/l</td>
<td>0.06 (0.04, 0.07)</td>
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<td>Insulin (FTHA study), pmol/l</td>
<td>4.3 (3.4, 5.6)</td>
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<td>75 (66, 85)</td>
<td>74 (66, 81)</td>
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All values are model-based means (95% CI); n, no. of subjects. Group P value indicates level differences over the study. Training effect indicates whether there is mean change between Pre and Post. Training × group describes whether mean changes are different between study groups. FFA, plasma free fatty acid concentration; FTHA, 14(R,S)-[18F]fluoro-6-thia-heptadecanoic acid; FDG, 2-deoxy-2-(18F)fluoro-D-glucose. FTHA study was measured in the fasting state during free fatty acid uptake measurements; FDG study was measured under euglycemic hyperinsulinemic clamp during glucose uptake measurements. Significant differences are in bold.

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Table 1. Characteristics and training adaptations in the SIT and MIT study groups

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Glucose, FFA, and Insulin AUC during OGTT

Insulin AUC in OGTT 3,842 (2,993, 4,934) 4,707 (3,599, 6,155) 4,774 (3,718, 6,129) 4557 (3509, 5917) 0.33 0.57 0.13

Glucose AUC in OGTT 824 (746, 902) 869 (783, 955) 878 (799, 956) 910 (827, 993) 0.19 0.35 0.82

Significant differences are in bold.
muscles \( (P = 0.23) \) and did not significantly respond to training \( (P = 0.82) \). FFAU was also analyzed in the individual muscles of the QF muscle group (Fig. 3, C and D). Both groups showed a similar \( (P > 0.5 \) in all comparisons between vasti muscles \) lack of response in vasti muscles, but, corresponding with GU results, the training response differed significantly \( (P < 0.048 \) in RF compared with all vasti muscles \) between the groups in RF compared with vasti muscles. While mean FFAU increased in RF by 10% in SIT, it decreased 20% in MIT.

**DISCUSSION**

In the present study, the effects of SIT and continuous MIT on glucose and fatty acid uptake were comprehensively investigated in different muscles in healthy middle-aged men. The main results show that, although upper-body muscles are activated during Wingate-type exercise (24), insulin-stimulated GU in the upper-body muscles did not increase in response to SIT training. Insulin-stimulated GU increased significantly.

**Fig. 1.** Examples of glucose uptake PET images in one subject from sprint interval training (SIT) group (left) and one subject from moderate-intensity training (MIT) group (right) before (top) and after (bottom) training interventions. Color bar on the left shows the glucose uptake rate, with red and yellow colors defining the areas of highest uptake.

**Fig. 2.** Skeletal muscle glucose uptake (A and B) and free fatty acid (FFA) uptake (C and D) in lower- and upper-body muscles. A and C: results from SIT group. B and D: results from the MIT group. Open bars show before, and shaded bars show after the training intervention. QF, quadriceps femoris; HAM, hamstrings; DEL, deltoids; BIC, biceps brachii; TRI, triceps brachii. Values are model-based means (95% confidence interval). *\( P < 0.001 \) for the time effect (pre- vs. posttraining comparison).
only in the QF muscle group. Interestingly, all four muscles of QF responded similarly to SIT, but only vasti muscles responded to MIT, while RF completely lacked the response to MIT. This reflects most likely the different activation pattern in this biarticular muscle in terms of SIT vs. MIT, and an inability of moderate-intensity exercise to effectively activate it during cycling exercise.

Muscle-Specific GU in Response to Training

GU was measured during euglycemic hyperinsulinemic clamp to simultaneously investigate whole body and muscle-specific GU. Whole body insulin-stimulated GU increased on average 12% by SIT and 8% by MIT without significant difference in training response between the groups. The observed change here is only modest compared with ~90% higher whole body insulin-stimulated GU observed in young highly trained endurance-athlete men compared with untrained men in a previous cross-sectional study (34). On the other hand, it should be noted that, in the present study, this was achieved with healthy middle-aged men in only 2 wk of training. In addition, it should be noted that whole body insulin-stimulated GU and fat-free mass increased and fat percent decreased comparably in both groups, despite that the calculated energy consumption during training was many fold higher in the MIT than SIT group. This makes an interesting addition to the findings of the recent study by Rosenkilde and colleagues (30), where they showed that the loss of weight and fat mass were not further enhanced when the time and energy consumption of moderate-intensity exercise training was doubled. Taken together, these findings suggest that, for the training benefits, training intensity is more important than energy consumption during training. This may be related to the fact that very high-intensity exercise seems to suppress postexercise energy intake compared with moderate-intensity exercise (33), but further studies are warranted. The gain in improvement would most probably be larger with prolonged training, as also supported by a recent study by Reichkendler and colleagues (28), showing ~30% increase in whole body insulin sensitivity after 11 wk of aerobic training. Nevertheless, our findings may be practical, particularly for the promotion of exercise as a prescription to improve whole body insulin sensitivity, as changes can be observed in such a short time.

As skeletal muscle is the main tissue responsible for glucose disposal during insulin stimulation (29), it was expected to find roughly corresponding changes, but larger in magnitude, were also observed in the main working muscles (QF muscle group as a whole) engaged in cycling training. Interestingly, much smaller and not statistically significant training-induced changes were observed in the hamstring muscles, and the changes were absent in the upper body muscles. A recent study showed that GU increased also in other thigh muscles than QF after 11 wk of aerobic training (28). In addition to a much
longer training period than in the present study, the subjects did the training in free-living conditions and also used different training modes than cycling (e.g., walking and running). Use of combination of cycling, walking, and jogging training may explain the differences compared with the present study, as previous PET studies show that GU increases more in anterior than posterior thigh muscles during cycling (13), while the response seems to be opposite in running (35).

When the training responses were compared among the five different large muscles of legs and arms in the present study, QF showed a much larger training response compared with all other muscles and without a significantly different response between the training modes. This is against our hypothesis, which expected that SIT would induce a larger increase in GU in upper body muscles. The reason for this is unclear, as other studies show that upper body muscles are mechanistically activated during the Wingate test (24), the protocol used in a repeated manner for training also in the present study. It is plausible that, even if arm muscles are heavily activated during sprinting activity, due to a fairly long and inactive recovery period overall, activity is not high enough for major improvements in metabolism. Nevertheless, this major finding strongly points to the conclusion that training-induced improvements in whole body and skeletal muscle insulin-stimulated GU are largely, although not necessarily solely if the training period is long enough, confined to those muscles that are strongly used during exercise (8). These findings also suggest that upper body muscles should be specifically trained if increased GU in those muscles, particularly, and maximal improvement at the whole body level are the aim. In this respect, it would be interesting to compare the responses that were observed in this present study for upper arm cycling or another exercise that really strains the upper body muscles.

The used PET method elegantly allowed us to investigate in detail the responses in different muscles of QF muscle group, and we found interesting results. Previous studies have shown that RF has low GU or FFAU and blood flow both at rest and during exercise (13, 15, 16, 18), and this was confirmed also in the present study. Interestingly, the training response was different in RF between the groups. While SIT effectively improved GU in all four heads of QF (including RF), the training response was completely absent in RF in MIT. There are at least two possible explanations for this.

First, despite the fact that QF muscles do not differ so much in fiber-type distribution in humans as in animals, RF has a usually larger proportion of type II muscle fibers than VL, VI, and VM (17). It is well known that type I fibers are predominantly activated at lower contraction intensities, and type II fibers are recruited significantly only at higher intensities or when type I fibers are fatigued or have depleted glycogen stores (12). Thus it may be that the differences in fiber-type distribution partly explain the completely absent training effect in RF in MIT. However, the results from a recent study argue against this explanation, as it was shown that different fiber types respond similarly to endurance type and SIT protocols (32).

Second, a major difference is that, contrary to the three vasti muscles, which participate in knee extension, RF is a biarticular muscle and contributes to both hip flexion and knee extension. It has been shown that, during both knee extension and hip flexion, RF is activated only at relatively high force levels (36). Even more relevant to this present study are the findings by Chin and colleagues (5) showing that, during cycling with different aerobic exercise intensities, normalized integrated-EMG in RF is at a much lower level than in VL and VM. In the same study, the authors measured also a near-infrared spectroscopy-derived deoxyhemoglobin signal that reflects microvascular \( \text{O}_2 \) extraction. The normalized oxyhemoglobin signal differed markedly between the muscles during moderate exercise intensities, being significantly lower in RF than two other muscles between 35 and 80% work rates (5).

Taken together, the findings described above and our findings in the present study suggest that the contribution of RF into the force production during moderate-intensity cycling is small and most probably mainly explains the poor training response to MIT in that muscle. It is interesting to note in this regard also the findings in the hamstrings. Although no statistically significant changes were observed, also in these biarticular muscles, the responses into the training modes differed much, showing a 17% increase in the group mean in SIT and a decrease of 7% in the group mean in MIT. It is known that, during low- and moderate-intensity exercise, hamstrings are not much activated (10), but, during faster cycling (19) and especially during Wingate sprint (10), the activation of this muscle group is increased.

**Muscle-Specific FFAU in Response to Training**

To measure muscle FFAU, we performed the PET studies when the subjects were in the fasting state, which is when the FFAU is usually highest during daily life. In contrasts to GU, we did not find any statistically significant changes in FFAU in any of the five larger muscles studied, neither after SIT nor after MIT. This is in accordance with our laboratory’s previous study in monozygotic twins discordant for physical activity and fitness, which showed no differences in QF FFAU between more and less active and fit brothers (15). Unfortunately, quite a few of the FTHA-PET measurements in the present study were unsuccessful due to technical reasons (total of \( n = 23/28 \) completed in pre- and \( n = 16/26 \) completed in postintervention measurements), which naturally reduced the power to detect the differences. However, with the use of hierarchical linear mixed model in the analysis, we could use the data from those who had missing values, thus improving the statistical power. This way, these findings should represent truly physiological effects rather than lack in statistical power.

When FFAU in the individual QF muscles was analyzed, we observed the same as with GU: a similar response in the vasti muscles between the training modes, but a different response in RF. Taken together, these findings support the idea that there are fundamental differences in training responses between RF and the other three QF muscles between moderate vs. sprint interval exercise and training.

**Study Limitations**

This study was performed in nonobese, middle-aged, untrained men, and the findings cannot, therefore, be directly extrapolated to other groups. The number of subjects was also relatively small, but on the other hand fairly typical for labor-demanding and detailed exercise physiological experiments and training interventions.
Conclusions

The results of the present study in healthy middle-aged men show that SIT and MIT with cycling exercise increase insulin-stimulated GU only in the QF muscle group and not in the hamstring or in the upper-body muscles. When analyzed in more details within the QF, RF did not respond to MIT, although it responded to SIT correspondingly as vasti muscles. This is most probably explained by poor activation of biarticular RF during moderate-intensity cycling. Taken together, these results emphasize and confirm previous findings with aerobic training (8) that, during MIT and SIT training, insulin-stimulated GU is improved only in those muscles that are significantly engaged in the power production during exercise.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS


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