Evaluation of individual skeletal muscle activity by glucose uptake during pedaling exercise at different workloads using positron emission tomography

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postexercise activity or muscle mass recruited. Romijn et al. (23) also described that tissue uptake of plasma glucose increases in relation to exercise intensity. Our group (7) previously reported that glucose uptake of active skeletal muscle during pedaling exercise increased significantly from 30% of maximal oxygen consumption (VO2max) to 55% VO2max intensity among untrained men. These results suggest that glucose uptake of skeletal muscle up to 55% VO2max intensity closely reflected muscle activity. In addition, Pappas et al. (21) stated that [18F]fluorodeoxyglucose (FDG) positron emission tomography (PET) successfully demonstrated increases in glucose consumption with increasing work production. Our group (5, 6, 25) reported on glucose uptake in individual skeletal muscles during aerobic running using PET. Similarly, Oi et al. (20) revealed lower extremity activity during level walking using PET. However, there are no reports observing individual skeletal muscle activities using PET during other sports or daily human activities. It is generally known that the quadriceps femoris muscle is the prime muscle during pedaling exercise, but details on the activities of other muscles, especially muscles deep in the body, are unknown.

In addition, previous studies have not demonstrated the effects of exercise intensity on the cooperative activity of individual skeletal muscles in the whole body. For example, the relative contribution of individual skeletal muscles during different levels of exercise intensity likely differs, even when the same exercise is being performed. Our group (7) observed that glucose uptake of muscles in the posterior part of thigh increased abruptly at high-intensity pedaling exercise compared with uptake at lower intensity exercise. This result indicated that the muscle activity of the posterior part of thigh would increase only at high intensity during pedaling exercise. However, there are no data regarding the relationship between individual muscle activities and exercise intensity on the whole body.

We hypothesized that muscles deep in the body might be activated during pedaling exercise and that the relative contribution of individual skeletal muscles during exercise might change when exercise intensity is increased. The purpose of the present investigation was to assess the muscle activities from the glucose uptake during pedaling exercise and to study the effect of exercise intensity (40 and 55% VO2max) on contributions of individual skeletal muscles for the exercise by using FDG and PET. Pedaling exercise was chosen because it is a
common daily movement and is also used worldwide for health promotion and various studies.

**MATERIALS AND METHODS**

**Subjects.** Twenty male subjects participated in this study. Subjects were healthy as judged by their medical history, physical examination, and routine laboratory tests. Subjects were randomly divided into two groups to limit the number exposed to the radioactive dosage of FDG: the exercise group (n = 7) and the control group (n = 13). The ethics committee of Tohoku University Graduate School of Medicine approved the study protocol. The purpose and potential risks were explained to all subjects, and written informed consent was received from all subjects before participation. The study was performed according to guidelines of the Declaration of Helsinki. The protocol of this study was approved by the ethics committee of Tohoku University Graduate School of Medicine, which is organized in accordance with the guidelines of the Ministry of Health, Labour, and Welfare of Japan.

**Study protocol.** Each exercise subject was studied on 2 separate days during a 3-wk period; study days were at least 2 days apart. All subjects could ride a bicycle, but were not athletes cyclists. Subjects fasted for at least 6 h before the study, and any kind of strenuous physical activity was prohibited for at least 1 day before the experiment. Teflon catheters were inserted in the forearm antecubital veins for injection of FDG and arterialized venous blood samplings. Plasma glucose, serum insulin, and blood lactate samples were drawn immediately before and at the end of exercise. Exercise subjects cycled (828E ergometer; Monark, Varberg, Sweden) at intensities of 40 or 55% VO_{2max} at 60 revolutions per minute (rpm) without a toe clip. The seat height for each subject was set so that the angle of the subject's knee joint was about 10° when the pedal was at the bottom dead center of the crank cycle. The order of exercise intensity was randomized. Before the experiment, all subjects were requested to rest on a bed for 20 min. After 10 min of exercise, FDG (49.3 ± 1.2 and 39.5 ± 2.8 MBq for 40 and 55% VO_{2max} exercise, respectively) was injected, and thereafter the exercise continued for 25 min, for a total exercise time of 35 min. For the control group, FDG (46.8 ± 19.4 MBq) was injected after 20 min of rest, and subjects continued to rest for an additional 25 min. Fifteen minutes after the end of exercise or rest, a whole body three-dimensional (3-D) static emission scan was performed.

**PET tracer, image acquisition, and processing.** The synthesis of FDG was done using an automated method described previously (11, 17). The specific radioactivity of synthesized FDG was >37 MBq/μmol, and the radiochemical purity exceeded 98%.

PET (SET 2400W; Shimadzu, Kyoto, Japan) with an intrinsic spatial resolution of 3.9 mm full width at half maximum was used to create a PET scan constructed of 13 frames from the foot to the head (8). Each frame took 180 s for a total of 39 min. All PET data were corrected for dead time, decay, and photon attenuations. A whole body transmission scan was performed with a 300 Ge/PbGa external rotating line source (370 MBq at purchase) maintaining similar procedures with PET emission scans. The axial field of view of the PET scanner was 200 mm. The image reconstruction was done by Tohoku University Supercomputer SX33 (NEC, Tokyo, Japan) through a 128 × 128 × 63 matrix for a set of 3-D volume images using a 3-D filtered back-projection algorithm (19).

**Biochemical analysis.** Measurements of glucose and lactate were done using the glucose oxidase method and a glucose analyzer (Glucose card GT-1630; KDK, Kyoto, Japan) and enzymatic lactate analyzer (Lactate Pro; KDK), respectively. In addition, serum insulin was measured using a double-antibody radioimmunoassay (Riabead 2; Dynabot, Tokyo, Japan); and γ coat cortisol; Incstar, Stillwater, MN).

**Magnetic resonance imaging.** After all PET scans, magnetic resonance imaging (MRI) scans were performed in anatomical locations similar to those of PET scans using the Spin Echo Sequence (Sigma; GE Yokogawa, Tokyo, Japan) at 1.5 tesla. The measurement conditions were as follows: repetition time/echo time, 320/20 ms; number of excitations, 3; field of view, 45 cm; number of matrix, 256 × 256; slice thickness, 10 mm; and gap between slices, 0 mm.

**Data analysis.** To analyze the regions of interest (ROIs), we obtained tomographic images at the level of the plantar, at the maximal girth of the leg (calf), at the midpoint of a line drawn between the patella and spina iliaca anterior superior (thigh), at the spina iliaca anterior superior (lower lumbar), at the 4th lumbar vertebra (upper lumbar), and at the 7th thoracic vertebra (chest). We measured FDG uptake in the 37 muscles in these regions. In addition, the location of individual skeletal muscles was confirmed by MRI (see Fig. 1C). To align the level in the PET image to the level in the MRI, we calculated the slice level from slice thickness and the slice number of each PET and MRI. These images were then displayed in isometry on the same screen. ROIs were drawn on PET images using image processing software (Dr. View; AJS, Tokyo, Japan).

The radioactivity from the 18F atom of the FDG molecule trapped in the skeletal muscle tissue, measured in counts per second (cps) per unit, indicates the degree of glucose uptake per unit volume. The glucose uptake per unit volume of skeletal muscle (cps/ml) of individual skeletal muscle, m indicates the total body weight in grams, d indicates the injected FDG dose, and f indicates the calibration factor. Detailed information is described in previous studies by our group (6, 25).

**Exercise capacity.** VO_{2max} was determined using direct measurement of oxygen consumption rate (AE300-SRC; Minato, Tokyo, Japan) and a bicycle ergometer (Monark 818EE) and was estimated using an incremental submaximal exercise test. Heart rate (HR) was monitored during exercise using a HR monitor (Vantage XL; Polar Electro, Kempele, Finland). The subjects cycled the bicycle ergometer at a pace of 60 rpm, with the load incrementally increased from 1 to 3 kp every 3 min. The estimated VO_{2max} was determined based on the relationships between HR–VO_{2max} and maximum HR (220 – age) (1).

**Statistical analysis.** All data are means ± SD. Statistical computations were performed with the SAS statistical program package (SAS Institute, Cary, NC). Student’s t-test, Welch’s t-test, or the Mann-Whitney U-test was used for analysis of statistical differences between the control group and exercise group at the intensity of 40% VO_{2max}. The Mann-Whitney U-test (nonparametric analysis) was used when data did not have normality. The paired t-test or Wilcoxon signed-rank test was used for the analysis of statistical differences between the exercise group at 40 and 55% VO_{2max} intensities. An analysis of variance followed by the Bonferroni/Dunn test (1-factor ANOVA) was used to compare the SUR of individual muscles in the quadriceps femoris muscle. The paired t-test was used for the analysis of statistical differences between post- and preexercise values of blood glucose, serum insulin, and plasma lactate. The significant differences for all data were set at P < 0.05, P < 0.01 and P < 0.001.

**RESULTS**

There were no significant differences in individual physical characteristics between exercise and control subjects (Table 1). Figure 1A illustrates typical whole body PET images after exercise at 40 and 55% VO_{2max} intensity. Figure 2 shows the mean SURs of the muscles of control subjects and exercise subjects at 40 and 55% VO_{2max} exercise intensity. At 40% VO_{2max} exercise intensity, SURs of the skeletal muscles in the
Table 1. Physical characteristics of subjects in each experimental group

<table>
<thead>
<tr>
<th></th>
<th>Control Group</th>
<th>Exercise Group</th>
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<tbody>
<tr>
<td>No. of subjects</td>
<td>13</td>
<td>7</td>
</tr>
<tr>
<td>Age, yr</td>
<td>23.3±3.7</td>
<td>22.9±4.1</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>61.9±6.7</td>
<td>61.1±7.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.3±1.9</td>
<td>20.6±2.5</td>
</tr>
<tr>
<td>[V\textsubscript{O}\textsubscript{2max}], ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>42.3±6.7</td>
<td>46.2±5.8</td>
</tr>
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</table>

Values are means ± SD. [V\textsubscript{O}\textsubscript{2max}], maximal aerobic power.

The purpose of the present investigation was to use FDG and PET to evaluate individual skeletal muscle activity based on glucose uptake during pedaling exercise at different workloads. FDG uptake of the iliacus muscle and the muscles in the anterior part of thigh was significantly higher in exercise (Fig. 2C). However, there were no significant increases in SURs of the psoas major, rectus femoris, tibialis anterior, tibialis posterior, and extensor digitorum longus muscles at 55% V\textsubscript{O}\textsubscript{2max} compared with values at 40% V\textsubscript{O}\textsubscript{2max} exercise intensity.

Figure 1, B and C, illustrates a typical PET image at the thigh level at both exercise intensities and an MRI obtained from the same region, respectively. The lower FDG uptake by the rectus femoris is shown in the PET image. The mean SURs of the rectus femoris were significantly lower than those of the vastus medialis and vastus intermedius at 40% V\textsubscript{O}\textsubscript{2max} exercise intensity and significantly lower than those of the other three muscles of the quadriceps femoris muscle at 55% V\textsubscript{O}\textsubscript{2max} exercise intensity (P < 0.05 or P < 0.01) (Fig. 2, B and C).

In the control subjects, the mean SURs of rectus femoris were lower than those of the vastus medialis and vastus intermedius (Fig. 2A).

No changes were observed in plasma glucose concentrations in subjects during exercise at 40 or 55% V\textsubscript{O}\textsubscript{2max} or in controls (Table 2). Compared with prerest or exercise values, serum insulin concentrations decreased slightly but significantly in the control group and the two exercise groups at postrest or exercise (P < 0.05). Plasma lactate concentrations remained unchanged during the control and exercise periods at 40% V\textsubscript{O}\textsubscript{2max} intensity but increased significantly during intensity levels of 55% V\textsubscript{O}\textsubscript{2max} (P < 0.01).

**DISCUSSION**

The purpose of the present investigation was to use FDG and PET to evaluate individual skeletal muscle activity based on glucose uptake during pedaling exercise at different workloads. FDG uptake of the iliacus muscle and the muscles in the anterior part of thigh was significantly higher in exercise...
subjects than control subjects. These results suggest that activities in the iliacus muscle and the muscles in the anterior part of the thigh increased during pedaling exercise. This is the first report that demonstrates increases in iliacus muscle activity during pedaling exercise.

Our group (7) previously reported that glucose uptake of skeletal muscle at up to 55% $\dot{V}O_2\text{max}$ intensity closely reflected muscle activity assessed by PET. Therefore, results of the present study imply that the iliacus muscle and the muscles in the anterior part of thigh are prime muscles for pedaling exercise. The iliacus muscle plays a role in hip-joint flexion, and muscles in the anterior part of the thigh function during knee-joint extension. During level walking and running, our previous results showed that the glucose uptake of the lower leg muscles was higher than that of the thigh muscles; however, the iliacus muscle did not have higher glucose uptake compared with the control state (6, 20). These results imply that pedaling exercise may be more useful for training the iliacus muscle than level walking and running. Because the iliacus muscle contributes to deep flexion of hip joint against the leg weight, this muscle plays an important role during step movements that occur in daily life. Whitt (27) has demonstrated that oxygen consumption for cycling at 12 miles/h (corresponding to active cycling speed in daily movement) with a touring (not racing) bicycle is 1.2 l/min. The oxygen consumption almost corresponds to 40% $\dot{V}O_2\text{max}$ of the present subjects of normal fitness level. In addition, 55% $\dot{V}O_2\text{max}$ of the present subjects ($\sim 1.55$ l/min) corresponds to the oxygen consumption for cycling on a road with 1% rising gradient (existing everywhere) at the same speed (12 miles/h) with the same bicycle. These values mean that anyone has an opportunity to ride a bicycle in these work intensities in daily life and that riding a bicycle with this speed and gradient might be a better countermeasure to decrease the risk of falling accident than level walking and running. Therefore, compared with training by level walking and running, training by pedaling exercise might be a better countermeasure to decrease the risk of falling accident.

Glucose uptake of the psoas major muscle, muscles in the posterior part of the thigh, and some muscles of the leg below

### Table 2. Changes in circulating substrate concentrations in each experiment

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th></th>
<th>Exercise</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>40% $\dot{V}O_2\text{max}$</td>
<td>Pre</td>
<td>Post</td>
</tr>
<tr>
<td>BG, mg/dl</td>
<td>92.6±7.7</td>
<td>91.2±10.9</td>
<td>100.1±10.9</td>
<td>94.1±12.4</td>
<td>99.3±8.5</td>
</tr>
<tr>
<td>SI, mU/l</td>
<td>5.4±3.0</td>
<td>4.3±1.9*</td>
<td>4.1±1.1</td>
<td>3.0±0.8*</td>
<td>4.4±1.5</td>
</tr>
<tr>
<td>La, mM</td>
<td>1.1±0.2</td>
<td>1.0±0.2</td>
<td>1.0±0.4</td>
<td>1.1±0.2</td>
<td>0.9±0.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; $n=13$ control subjects and 7 exercise subjects. Pre and post represent pre- and postrest values. BG, blood glucose level; SI, serum insulin level; La, plasma lactate level. *$P<0.05$; †$P<0.01$ vs. Pre value in each experiment.
the knee joint also increased significantly at 40% \( \text{VO}_{2\text{max}} \) exercise intensity compared with those of control subjects. These results indicate that these muscles play important roles in pedaling exercise. The psoas major muscle plays a role in hip-joint flexion, and biceps femoris, semitendinosus, and semimembranosus hamstring muscles act during hip-joint extension. The adductor magnus, adductor, and gracilis muscles play a role in knee-joint extension and/or flexion during pedaling exercise. Leg muscles below the knee joint function with ankle joint extension and/or flexion. Takahashi et al. (24) clarified that the decline of the psoas major muscle starts at ages in the 20s and that in women, the decline in this muscle started earlier than the decline in the quadriceps femoris muscle. In addition, the decline of the psoas major muscle from ages in the 60s through the 70s was most remarkable. They also have shown that the psoas major muscle plays an important role in the walking ability of elderly persons and that declines in this muscle may contribute to falling. Therefore, falling accidents of elderly persons during walking may possibly be reduced by starting pedaling training early.

At 55% \( \text{VO}_{2\text{max}} \) exercise intensity, glucose uptake of the iliacus muscle and muscles in the thigh, except for the rectus femoris, increased significantly compared with those at 40% \( \text{VO}_{2\text{max}} \) exercise intensity \((P < 0.05, P < 0.01, \text{or } P < 0.001)\). There was, however, no significant increase in glucose uptake of the psoas major, rectus femoris, and muscles of the leg below the knee joint at 55% \( \text{VO}_{2\text{max}} \) compared with those at 40% \( \text{VO}_{2\text{max}} \) exercise intensity. These results suggest that the activated muscles during pedaling exercise, with the exclusion of the psoas major, rectus femoris, and the muscles of the leg below the knee joint, contribute to the increase of workload from 40 to 55% \( \text{VO}_{2\text{max}} \). This result is also a novel finding in this study. The iliacus muscle and psoas major muscle are often considered a single muscle, referred to as the iliopsoas muscle. However, our results indicate that the iliacus muscle contributed to the increase of workload in the pedaling exercise more than the psoas major muscle.

This difference in function of the iliacus muscle and psoas major muscle might be caused by anatomical differences between these muscles. The iliacus muscle can directly affect the force to the thigh, since the iliacus muscle is a monoarticular muscle that crosses only the hip joint. However, the psoas major muscle is a multiarticular muscle that crosses the lumbar vertebrae joint and hip joint. For full multiarticular muscle activation, either the origin of the muscle or the insertion of the muscle, or both, must be fixed. The activity of the psoas major muscle during pedaling exercise at 55% also might be lower based on movement of the lumbar vertebrae joint due to the increase in exercise intensity; however, we cannot elucidate the clear cause of the difference in the activities of these muscles during different exercise intensities in this study.

Our results demonstrate that the activity of the rectus femoris is lower than that of other quadriceps femoris muscles during pedaling exercise. The glucose uptake of the rectus femoris muscle was significantly lower than that of the other three muscles in the quadriceps femoris muscle during pedaling exercise. In the control subjects, the glucose uptake of the rectus femoris muscle was lower than those of the vastus medialis and vastus intermedius muscle. The glucose uptake of skeletal muscle is affected by muscle fiber distribution. The vastus medialis muscle has a slightly higher number of type I fibers than rectus femoris muscle, but the vastus lateralis and rectus femoris muscle have a similar muscle fiber distribution (15). We do not have data on the muscle fiber distribution in vastus intermedius muscle. Therefore, the one of the causes of the difference in glucose uptake between vastus medialis muscle and rectus femoris muscle at pedaling exercise may be the slightly different muscle fiber distribution in this study. However, the differences in glucose uptake between vastus lateralis and rectus femoris muscle also cannot be explained solely by differences in muscle fiber distribution at 55% \( \text{VO}_{2\text{max}} \) intensity. Ericson et al. (4) reported that peak activity of the rectus femoris on the EMG was significantly lower than that of the vastus medialis and vastus intermedius during pedaling exercise. Endo et al. (3) also demonstrated the lower activity of the rectus femoris during pedaling exercise at some intensity, using MRIs. The data in our study correspond with their results.

This difference in function of the rectus femoris muscle and the other three muscles of quadriceps femoris muscle might be caused by anatomical differences between these muscles. The other three muscles can directly affect the force to the tibia, because they are monoarticular muscles, crossing only the knee joint. However, the rectus femoris muscle is a multiarticular muscle, crossing at the hip joint and knee joint. During pedaling exercise, the movement of hip joint and knee joint occur simultaneously. This simultaneous movement of two joints might explain the lower activity of the rectus femoris muscle during pedaling exercise at <55% \( \text{VO}_{2\text{max}} \) intensity.

Factors that influence the glucose uptake of skeletal muscles include muscle fiber distribution (12–14), muscle activity, defined as the power output, plasma glucose levels, and insulin concentrations. However, the large differences in glucose uptake among the muscles noted in this study cannot be explained only by differences in muscle fiber distribution as described in previous studies (7, 16). A previous study demonstrated that the difference in muscle fiber distribution is observed in many muscles (15). However, the percentage difference between type I fibers and type II fibers is <20% in most muscles with the exception of a few, such as the soleus and tibialis anterior. Gaster et al. (10) reported that the difference in density of glucose transporter 4 between slow fibers and fast fibers was ~12% in young subjects. Thus the big differences in \( \text{SUR} \) among the muscles in this study cannot be explained solely by differences in muscle fiber distribution. Although the blood flow in muscle is thought to be one factor that affects glucose uptake by muscles (2), one study (19) strongly supported the idea that glucose and FDG uptake are not regulated by blood flow. In the present study, blood glucose concentrations did not change, and the serum insulin concentrations decreased significantly in the control and exercise groups. These results suggest that changes in serum insulin levels did not influence FDG uptake in this study.

Currently, PET scanning during dynamic exercise is technically problematic. Therefore, the injection of FDG as used in the present study was performed during exercise, which was then continued for an additional 25 min, and scanning was performed after exercise. Despite this, the measured glucose uptake values reflect actual glucose uptake during exercise. After phosphorylation, the glucose analog (FDG) is trapped inside the muscle cell where further metabolism is prevented due to its chemical characteristics. This metabolic state of
phosphorylated FDG is preserved for ~2 h after injection (9). Plasma radioactivity peaked shortly after FDG injection and then decreased quickly during exercise (16). In addition to the tissue glucose uptake, the total uptake of tracer into the tissues is dependent on the availability of the tracer.

In this study, subjects were not bicycle race athletes, and we used a touring (not racing) bicycle ergometer. Therefore, the results of this study demonstrate muscle activities representative of subjects who have ordinary pedaling skills. Results might be different among bicycle race athletes, who have a higher level of pedaling skills.

One limitation of this study was the use of a separate control and exercise group as opposed to a crossover design. This was done to limit the radioactive dosage for each subject much as possible, based on the recommendations of the ethical committee of Tohoku University Graduate School of Medicine.

In conclusion, results of this study suggest that the activity of the iliacus muscle and muscles of the anterior part of thigh were critical during the pedaling exercise. The muscles in the posterior part of the thigh are important for pedaling exercise, although activation levels are lower than for the iliacus muscle and muscles of the anterior part of thigh. In addition, the iliacus muscle and all muscles in the thigh, with the exception of the rectus femoris and psoas major muscle, contribute to increasing workload of pedaling exercise from 40 to 55% VO_{2max} intensity.

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