Muscle oxygen extraction and perfusion heterogeneity during continuous and intermittent static exercise

KARI K. KALLIOKOSKI,1 MARKO S. LAAKSONEN,1 TEEMU O. TAKALA,1,2 JUHANI KNUUTI,1 AND PIRJO NUUTILA1,2

1Turku Positron Emission Tomography Centre, and 2Department of Medicine, University of Turku, FIN-20521 Turku, Finland

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Kalliokoski, Kari K., Marko S. Laaksonen, Teemu O. Takala, Juhani Knuuti, and Pirjo Nuutila. Muscle oxygen extraction and perfusion heterogeneity during continuous and intermittent static exercise. J Appl Physiol 94: 953–958, 2003.—The purpose of this study was to investigate the effects of different forms of static exercise on muscle perfusion, perfusion heterogeneity, and oxygen extraction. Perfusion and oxygen uptake of quadriceps femoris muscle were measured in 10 healthy men by using positron emission tomography and [15O]H2O and [15O]O2 first during intermittent static exercise (10% of maximal static force (MSF)) and thereafter during continuous static exercise at the same tension-time level (5% static; 5% of MSF). In 4 of these subjects, perfusion was measured during continuous static exercise with 10% of MSF (10% continuous) instead of the second [15O]O2 measurement. Muscle oxygen consumption was similar during intermittent and 5% continuous, but muscle perfusion was significantly higher during 5% continuous. Consequently, muscle oxygen extraction fraction was lower during 5% continuous. Perfusion was also more heterogeneous during 5% continuous. When exercise intensity was doubled during continuous static exercise (from 5% continuous to 10% continuous), muscle perfusion increased markedly. These results suggest that continuous, low-intensity static exercise decreases muscle oxygen extraction and increases muscle perfusion and its heterogeneity compared with intermittent static exercise at the same relative exercise intensity.

BLOOD FLOW IS AN IMPORTANT FACTOR regulating oxygen supply of skeletal muscle during exercise. Early studies have shown that continuous static exercise hinders muscle perfusion because of increased intramuscular pressure (2, 21). At higher static exercise intensities (>10–15% of maximal voluntary contraction), impaired muscle perfusion and oxygen delivery are believed to be the major explanations for muscle fatigue (10, 23). At lower intensities (<10–15% of maximal voluntary contraction), muscle perfusion has been shown to remain sufficient to deliver enough oxygen, but marked muscle fatigue during prolonged exercise has been shown to still occur (23). It has been suggested that the fatigue might be related to an increase in muscle water content in interstitial space, which increases diffusion distance and may impair substrate extraction from interstitium (23). Another potential factor causing impaired substrate extraction could be heterogeneity in substrate delivery. Recent studies have shown that increased blood flow heterogeneity is associated with impaired substrate extraction in peripheral tissues (14, 26). However, the effects of static continuous exercise on muscle perfusion heterogeneity are unknown.

Thus the purpose of the present study was to investigate the effects of different forms of static exercise on perfusion heterogeneity and its association to muscle oxygen extraction. Muscle perfusion, its heterogeneity, and muscle oxygen consumption and oxygen extraction were measured by using positron emission tomography (PET) with [15O]H2O and [15O]O2 as tracers during continuous and intermittent static exercise.

METHODS

Subjects. Ten healthy men were studied (age 26 ± 3 yr, body mass index 23 ± 3 kg/m2). Written, informed consent was obtained after the purpose, nature, and potential risks of the study were explained to the subjects. The Joint Commission on Ethics of the University of Turku and Turku University Central Hospital approved the study protocol.

Study design. Studies were performed after an overnight fast. Before PET studies, maximal static force (MSF) of the right knee extensors was measured with a dynamometer (KinCom, Chattex, Chattanooga, TN) as previously described (14). Two catheters were inserted, one in an antecubital vein for injection of tracers and one in the opposite radial artery for blood sampling. The subjects were positioned in supine position in the PET scanner, with the femoral regions in the gantry and the exercising leg fastened to dynamometer (I-KON, Chattanooga Group, Oxfordshire, UK) at a knee angle of 50°. Care was taken to fasten subjects carefully to the imaging table to avoid any movements in the femoral region during the study. Studies started with a resting period of 15 min, during which transmission scan for correction of photon attenuation was performed (Fig. 1). Thereafter, one-legged intermittent static exercise (2 s of exercise, 2 s of rest) with 10% of MSF (intermittent-10%) was performed for 60 min for

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Measurement of muscle oxygen consumption began by pumping the input curve and the tissue curve was solved by an automatic gamma counter (Wizard 1480, Scanditronix, Uppsala, Sweden) cross-calibrated with an MSF (continuous-10%). Radioactivity concentration was measured as described above in the perfusion measurements. Muscle oxygen uptake was calculated as previously described (15). Oxygen extraction fraction was calculated as O₂ uptake/ (perfusion·O₂ content in blood), where O₂ uptake was calculated from [¹⁵O]O₂ studies and perfusion from [¹⁵O]H₂O studies.

All PET data were corrected for deadtime, decay, and measured photon attenuation. PET images were processed by using a 2D-ordered subsets expectation maximization and median root prior reconstruction with 150 iterations and the Bayesian coefficient of 0.3 (1).

ROIs. ROIs surrounding quadriceps femoris (QF) muscle groups and individual muscles were drawn into four subsequent cross-sectional planes in both thighs, as previously described (12, 14). Localization of the muscle compartments was done on the basis of individual transmission and perfusion images. The voxel size in the present study was 3.1 x 3.1 x 6.75 mm (width x height x depth). Thus total thickness of the ROIs in four planes in proximal-distal direction was 2.7 cm. Total numbers of the voxels within the muscles were as follows: vastus lateralis 1,634 ± 264; vastus intermedius 524 ± 80; vastus medialis 422 ± 77; and rectus femoris 584 ± 124 voxels. Thus total volumes of the analyzed area were: vastus lateralis 106.0 ± 17.1 cm³; vastus intermedius 34.0 ± 5.2 cm³; vastus medialis 27.4 ± 5.0 cm³; and rectus femoris 37.9 ± 8.0 cm³.

Calculation of perfusion heterogeneity within muscles. Voxel by voxel perfusion data from four planes were pooled, and means ± SD of perfusion values were calculated. Relative dispersion (RD) of perfusion was calculated as RD = (SD/mean) x 100%, where SD is the standard deviation. Heterogeneity due to methodological factors estimated from phantoms, averaged 10% at the activity levels of the resting muscles and 6% at the activity levels of the exercising muscles with the median root prior reconstruction method (12).

Fractal analysis, as previously described (13), was used to assess fractal dimension of perfusion heterogeneity and perfusion heterogeneity in microvascular units. In brief, square ROIs (8 x 8 voxels) were placed into four cross-sectional planes of the QF muscle of the exercising leg. The ROI was placed over the vastus intermedius as well as possible. Thereafter, three-dimensional matrices of voxel perfusion values (256 voxels) were regrouped to larger groups by combining adjacent voxels in a predetermined manner to the level where eight combined pieces remained. Mean, standard deviation, and RD of perfusion were calculated after each regrouping. Heterogeneity due to methodological factors was estimated and subtracted from measured RD values as previously described (13). The logarithm of RD of perfusion vs. the logarithm of the number of the combined voxels was plotted, and the fractal dimension was calculated as k − 1, where k is the slope of linear least-square regression line of the data. Perfusion heterogeneity in microvascular units (1 mm³) (9) was estimated by using the measured heterogeneity in voxels (RD = SD/mean) and fractal dimension values (13).

Statistical methods. Statistical analyses were performed by using SAS/STAT statistical analysis software release 8.2 (SAS Institute, Cary, NC). Student’s paired t-test was used for the analysis of statistical differences in whole QF values between exercise modes. ANOVA for repeated measurements was used for the analysis of statistical differences between each subject, during which muscle perfusion and oxygen uptake were measured as described in detail in Measurements of muscle perfusion, perfusion heterogeneity, and oxygen uptake. Finally, muscle blood volume scan with [¹⁵O]CO was performed to help draw the regions of interest (ROIs) and to avoid areas of large vessels in the ROIs (17). After that, continuous static exercise with 5% of MSF (continuous-5%) was performed for 30 min, during which the measurements were repeated. Four subjects performed the last 15 min of the continuous exercise with the intensity of 10% of MSF (continuous-10%), and muscle perfusion was measured instead of oxygen uptake.

Measurements of muscle perfusion, perfusion heterogeneity, and oxygen uptake. Positron-emitting tracers [¹⁵O]H₂O and [¹⁵O]O₂ were produced as previously described (22, 24). An ECAT 931/08 tomograph (Siemens/CTI, Knoxville, TN) was used for image acquisition. For the femoral muscle perfusion studies, a 6-min dynamic scan (time frames of 6 x 5, 6 x 15, and 8 x 30 s) was performed immediately after an intravenous injection of 1.21 ± 0.17 GBq (32.7 ± 4.5 mCi) of [¹⁵O]H₂O. Input function was obtained from forearm arterial blood, which was continuously withdrawn with constant speed by using a pump. Radioactivity concentration was measured by using a two-channel, on-line detector system (Scanditronix, Uppsala, Sweden) cross-calibrated with an automatic gamma counter (Wizard 1480 3°, Wallac, Turku, Finland) and the PET scanner (16). The delay between the input curve and the tissue curve was solved by fitting, and muscle perfusion was calculated by the autoradiographic method (20) voxel-by-voxel into perfusion images by using a 250-s tissue integration time (16).

Skeletal muscle oxygen consumption was measured by a bolus inhalation technique previously validated against direct measurements of arteriovenous O₂ difference (15). Measurement of muscle oxygen consumption began by pumping [¹⁵O]O₂ into a rubber bladder and mixing it with room air. Thereafter, subjects inhaled the gas as a single bolus containing 1.14 ± 0.14 GBq (30.7 ± 3.8 mCi) of [¹⁵O]O₂. Dynamic PET imaging of the femoral region was started simultaneously and performed for 7 min with time frames of 6 x 5, 6 x 15, 6 x 30, and 2 x 60 s. The input function was measured as described above in the perfusion measurements. Muscle oxygen uptake was calculated as previously described (15). Oxygen extraction fraction was calculated as O₂ uptake/ (perfusion·O₂ content in blood), where O₂ uptake was calculated from [¹⁵O]O₂ studies and perfusion from [¹⁵O]H₂O studies.

Fig. 1. Study protocol. The 10 study subjects performed one-legged static exercise with intermittent and continuous modes. Arrows denote the injection of the tracers. Positron emission tomography (PET) scanning was performed after each injection. Four subjects performed the last 15 min of the exercise with 5% of MSF (continuous-5%) and muscle perfusion was measured instead of oxygen uptake.
individual QF muscles and exercise modes. Correlation in perfusion values between different modes of static exercise was calculated by using Pearson correlation coefficient. P values of <0.05 were considered statistically significant. All data are shown as means ± SD.

RESULTS

MSF and exercise during PET. MSF of the exercising leg was 646 ± 127 N. Tension during exercise with 10% of MSF was 64 ± 13 N, and during exercise with 5% of MSF, it was 32 ± 6 N.

Muscle oxygen uptake, perfusion, and perfusion heterogeneity. Muscle oxygen uptake was similar during both exercise modes (intermittent-10%: 5.9 ± 1.9; continuous-5%: 6.2 ± 1.9 ml·kg muscle⁻¹·min⁻¹; P = 0.599, n = 6; Fig. 2A). Muscle perfusion was higher (95 ± 26 and 147 ± 53 ml·kg muscle⁻¹·min⁻¹; P < 0.001, n = 10; Fig. 2C), and consequently, muscle oxygen extraction fraction was lower during continuous-5% (0.37 ± 0.22 and 0.30 ± 0.23; P = 0.036, n = 6; Fig. 2B). Also, the increase in muscle blood flow (2.9 ± 0.6- vs. 5.6 ± 2.9-fold, P = 0.006) from the resting values (35 ± 18 and 30 ± 14 ml·kg muscle⁻¹·min⁻¹; P = 0.17, n = 10) was significantly higher during continuous-5%. Muscle perfusion was more heterogeneous during continuous-5% in whole QF (RD of 56 ± 14 vs. 62 ± 14; P = 0.05, n = 10; Fig. 2D) but not significantly different in different muscles of QF (Fig. 3B). However, mean perfusion varied significantly between different regions and more during continuous-5% (Fig. 3A). Perfusion was significantly higher in vastus medialis and vastus intermedius parts of QF muscle group during continuous-5% than during intermittent-10%. In vastus lateralis and rectus femoris, no differences in perfusion were found between exercise modes. Fractal dimension of perfusion heterogeneity was similar between exercise modes (intermittent-10%: 1.16 ± 0.07; continuous-5%: 1.16 ± 0.07; P = 0.86, n = 10), as was also estimated perfusion heterogeneity in microvascular units (38 ± 5 and 37 ± 11; P = 0.56, n = 10).

When exercise intensity was doubled during continuous exercise, muscle perfusion increased from 140 ± 60 to 216 ± 44 ml·kg muscle⁻¹·min⁻¹ (Fig. 4). Two subjects (S3 and S4) doubled perfusion with the increased intensity of continuous exercise, whereas two others (S1 and S2) had only minor increases (~15%).

Correlation of voxel perfusion between measurements. Variable correlation was found between the voxel perfusion values during intermittent-10% and continuous-5% (Fig. 5). In some subjects, either areas with low perfusion during intermittent exercise increased perfusion during continuous exercise or vice versa, which caused a weaker overall correlation within QF. Good correlation was found between the

Fig. 2. Muscle oxygen uptake (A), oxygen extraction (B), perfusion (C), and perfusion heterogeneity (D, relative dispersion) during intermittent exercise with 10% of maximal force (intermittent 10%; open bars) and continuous exercise with 5% of maximal force (continuous 5%; filled bars). *P < 0.05 vs. intermittent static exercise.
voxel perfusion values in two levels of continuous static exercise in those two subjects who increased perfusion most, whereas in the other two subjects the correlation was weaker (Fig. 6).

**DISCUSSION**

The findings of the present study show that muscle perfusion is increased and oxygen extraction reduced during continuous compared with intermittent static exercise at the same workload. Furthermore, these changes were associated with increased perfusion heterogeneity within QF muscle group during continuous static exercise.

Workloads in the present study were chosen according to MSF test to represent 5% (continuous) and 10% (intermittent and continuous with higher workload) of MSF. It is not possible to measure work done during static exer-
cise, and, therefore, other estimates of workload were used. The intensities between 10% intermittent and continuous-5% exercise were chosen to produce the same tension-time index, which has been previously used to compare workloads during dynamic and static exercise (8) and to produce equal workloads. In addition, muscle oxygen consumption, a direct index of aerobic metabolism, was found to be similar during both intermittent and continuous static exercise. These both suggest that workloads were comparable between exercise modes. Interestingly, despite similar workload, perfusion was increased during continuous mode to provide enough oxygen.

In the present study, we tested whether continuous exercise affects perfusion heterogeneity and whether it is associated with changes in oxygen extraction in muscle tissue. The findings showed that perfusion is more heterogeneous in QF during continuous exercise, and this more heterogeneous perfusion is associated with impaired oxygen extraction. It has been previously suggested that an increase in muscle water content in interstitial space during continuous static exercise could be one potential reason for impaired substrate extraction from interstitium (23). The results of the present study suggest that perfusion heterogeneity could also be related to changes in substrate extraction during continuous static exercise. However, more heterogeneous perfusion in the whole QF muscle group during continuous exercise was mostly explained by increased differences in mean perfusion between different muscles of QF and not by changes in heterogeneity within individual muscles. Findings in fractal analysis also supported this. As expected, fractal dimension, which is a good measure of vascular branching pattern (11, 25), was not different between exercise modes. What is interesting, estimated perfusion heterogeneity in microvascular units was also similar between exercise modes. Therefore, we conclude that static exercise mode has effects on perfusion distribution between but not within individual muscles. It is worthy of note that the imaged area was in the middle of the thigh, and, therefore, these results represent only a limited area in the proximal-distal direction. It has been shown that there are differences in perfusion along the muscle in proximal-distal direction, but the limited area of the PET scanner makes it difficult to study this issue with PET. This is where the use of other methods, for example near infrared spectroscopy (3, 4), could have been useful.

The finding that perfusion during intermittent exercise was lower than during continuous exercise seems illogical at first glance. However, the exercise intensities were low, and it has been previously shown that muscle perfusion remains sufficient at intensities of <10% maximal voluntary contraction (23). The findings of the present study support this finding because perfusion was higher during continuous-5% than during intermittent static exercise and perfusion during continuous-10% than during continuous exercise with 5%.

In previous studies, changes in oxygen uptake without changes in blood flow by vasoactive substances have been regarded as markers of flow variation between nutritive and nonnutritive routes (5–7, 18). In the present study, we found the opposite: increased perfusion without any changes in oxygen uptake. This might indicate increased nonnutritive perfusion during continuous exercise. However, this issue remains to be further studied because it is presently impossible to differentiate perfusion between these two routes within muscle by using PET.

Strength of the PET method applied in the present study is the ability to measure perfusion distribution within the muscle. This enabled us to study regional correlation between muscle perfusion during different modes of static exercise. These analyses showed variable correlation in regional flows between intermittent and continuous static exercise modes. Six of ten subjects had almost an even increase in perfusion in all regions, as shown by good correlation between exercise modes in Fig. 4. However, the other four subjects had poorer correlation, suggesting that different regions were activated during intermittent and continuous static exercise. On average, vastus medialis and intermedius had significantly higher perfusion during continuous-5% than during intermittent-10% exercise, whereas in rectus femoris and vastus lateralis no differences were found. This suggests that the former two muscles were activated more during continuous-5% than during intermittent-10%, whereas the latter two were on average equally activated between exercise modes. It would have been nice to also correlate voxel perfusion and oxygen metabolism, but unfortunately it is presently impossible, or at least the results would not be accurate enough, to calculate oxygen consumption or extraction in the voxels. Thus there is definitely a need to develop the oxygen consumption method further so that the question of whether there is match or mismatch between voxel perfusion and oxygen extraction during intermittent exercise could be one potential reason for impaired substrate extraction from interstitium (23). The results of the present study suggest that perfusion heterogeneity could also be related to changes in substrate extraction during continuous static exercise. However, more heterogeneous perfusion in the whole QF muscle group during continuous exercise was mostly explained by increased differences in mean perfusion between different muscles of QF and not by changes in heterogeneity within individual muscles. Findings in fractal analysis also supported this. As expected, fractal dimension, which is a good measure of vascular
metabolism could be answered also in humans. Regarding this, a recent study by Richardson and colleagues (19) that applied MRI and MRS suggests that there is poor match between local blood flow and oxygen consumption. However, in that study, parameters were measured in much larger samples than the voxel size in the present study.

When the intensity was doubled during continuous static exercise, the mean increase in muscle perfusion was >50%, but the interindividual variation was large. Two of the four subjects failed to increase muscle perfusion at the exercise intensity of 10% of MSF. Interestingly, these two subjects also had a poor correlation between voxel perfusion values at two intensities of continuous static exercise. Evidently, they activated different muscles during these two intensities, but the reason for this is unknown. Perhaps fatigue that is observed during prolonged static exercise may be related to these individual differences in ability to increase muscle perfusion. Taken together, these correlation results suggest that there is large variation between subjects in muscle recruitment during intermittent and continuous static exercise and between exercise intensities during continuous static exercise.

In conclusion, these results show that muscle perfusion during low-intensity exercise is increased during continuous compared with intermittent static exercise at the same workload. Furthermore, the increase is associated with changes in muscle oxygen extraction and perfusion heterogeneity within the QF muscle group during continuous static exercise. Lower oxygen extraction may be one of the causes to muscle fatigue observed at the low level of continuous static exercise.

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