Anaerobic capacity and muscle activation during horizontal and uphill running

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Sloniger, Mark A., Kirk J. Cureton, Barry M. Prior, and Ellen M. Evans. Anaerobic capacity and muscle activation during horizontal and uphill running. *J. Appl. Physiol.* 83(1): 262–269, 1997.—Anaerobic capacity as measured by the maximal or peak oxygen deficit is greater during uphill than during horizontal running. The objective of this study was to determine whether the greater peak oxygen deficit determined during uphill compared with horizontal running is related to greater muscle volume or mass activated in the lower extremity. The peak oxygen deficit in 12 subjects was determined during supramaximal treadmill running at 0 and 10% grade. Exercise-induced contrast shifts in magnetic resonance images were obtained before and after exercise and used to determine the percentage of muscle volume activated. The mean peak oxygen deficit determined for uphill running [2.96 ± 0.63 (SD) liters or 49 ± 6 ml/kg] was significantly greater ($P < 0.05$) than for horizontal running (2.45 ± 0.51 liters or 41 ± 7 ml/kg) by 21%. The mean percentage of muscle volume activated for uphill running [73.1 ± 7.4% (SD)] was significantly greater ($P < 0.05$) than for horizontal running (67.0 ± 8.3%) by 9%. The differences in peak oxygen deficit (liters) between uphill and horizontal running were significantly related ($y = 8.05 \times 10^{-4}x + 0.35$; $r = 0.63$, SE of estimate = 0.29 liter, $P < 0.05$) to the differences in the active muscle volume (cm^3) in the lower extremity. We conclude that the higher peak oxygen deficit during uphill compared with horizontal running is due in part to increased mass of skeletal muscle activated in the lower extremity.

anaerobic metabolism; magnetic resonance imaging; skeletal muscle activation; oxygen deficit

ENERGY DEMANDS during exercise are met through aerobic and anaerobic processes. During the early stages of an exercise bout, there is an imbalance between the energy demand and the energy supplied aerobically. The difference between the energy demand and the measured oxygen uptake ($\dot{V}O_2$) at the onset of exercise was referred to by Krogh and Linhard (31) as the oxygen deficit and was used as a measure of anaerobic metabolism. The peak oxygen deficit measured during supramaximal exercise has been used to quantify the total energy yield from anaerobic processes, i.e., the anaerobic capacity (27).

There is a direct relationship between the peak oxygen deficit measured during supramaximal treadmill running and treadmill incline (36, 44). In addition, Walker et al. (44) demonstrated that the peak oxygen deficit measured during uphill supramaximal treadmill running was augmented if external weight was carried. The mechanism(s) responsible for these effects is unknown but has been hypothesized to reflect increased active muscle mass and/or altered running efficiency (36, 44). These hypotheses have not been tested.

In recent years, exercise-induced contrast shifts in magnetic resonance (MR) images have been used to determine the pattern and intensity of muscle activation during exercise (1, 2, 19, 20, 22, 23, 30, 38, 41, 46). Through the use of this relatively new technology, the relationship between the peak oxygen deficit and active muscle volume or mass can be determined. The objective of this study was to test the hypothesis that the greater peak oxygen deficit during uphill compared with horizontal running is related to greater muscle activated in the lower extremity.

METHODS

Subjects. The subjects were 12 physically active female college students. All subjects were involved in vigorous running for conditioning at the time of the study. Mean physical characteristics of the subjects were as follows: age, 24 ± 3 (SD) yr; mass, 59.7 ± 8.2 kg; percent fat, 20.0 ± 4.9%; and peak $\dot{V}O_2$ ($\dot{V}O_{2peak}$), 2.91 ± 0.52 l/min or 49 ± 5 $ml \cdot kg^{-1} \cdot min^{-1}$. Subjects received an explanation of the time commitment and procedures involved in the study. Each subject read and signed a consent form and completed medical history and training background questionnaires. The study was approved by the Institutional Review Board.

Testing procedures. Each subject completed seven test sessions on separate days. Three of the sessions were used to measure metabolic responses and muscle activation during horizontal running and three were used to obtain the same measures during uphill (10% grade) running. The order of the three horizontal and three uphill tests was balanced. During the first test session, subjects completed a discontinuous, speed-incremented treadmill test to exhaustion under one of the two conditions (horizontal or uphill). $\dot{V}O_{2peak}$ was determined, and part of the data needed to determine peak oxygen deficit was collected. To determine the oxygen cost of running at submaximal speeds, subjects first completed six 6-min submaximal bouts of treadmill running. Treadmill speed was increased in evenly spaced increments so that the sixth bout involved running at a speed estimated to be close to the maximal speed that the subject could maintain for 6 min. To determine $\dot{V}O_{2peak}$, additional bouts of running were completed in which treadmill speed was increased until the subject could not finish a 6-min bout. Treadmill speeds ranged from 7.9 to 17.1 and from 5.3 to 9.8 km/h for the horizontal and uphill conditions, respectively.

During the bouts of running, metabolic measurements were obtained by using a computer-automated system. The volume of inspired air was measured by a Rayfield mechanical flowmeter (model 9200, Rayfield Equipment, Waitsfield, VT). The concentrations of carbon dioxide and oxygen in the expired air were measured by Ametek CD-3A and S-A/I electronic gas analyzers, respectively. Standard gases analyzed by the micro-Scholander chemical gas analyzer were used to calibrate the analyzers before the test. One-minute averages of $\dot{V}O_2$ and other metabolic measures were calculated every 15 s by using modified Vista software (Rayfield Equipment). Maximal heart rate was determined by using a heart rate monitor (Polar Vantage XL). The highest $\dot{V}O_2$

obtained on the test was operationally defined as the $\dot{V}O_{2peak}$ if *1*) there was a plateau in $\dot{V}O_2$ between the last two stages of the test as assessed by an increase in $\dot{V}O_2$ of <2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ (43) or *2*) peak heart rate was at least 90% of age-predicted maximum and respiratory exchange ratio was >1.0.

During the second test session under each of the experimental conditions, subjects completed a supramaximal ($\sim$115% $\dot{V}O_{2peak}$) bout of treadmill running to exhaustion. Treadmill speed was set to elicit exhaustion within 2–4 min. The subject was required to run for at least 2 min for the test to be considered valid. The mean treadmill time for both conditions was 2.96 ± 0.4 min and ranged from 2.2 to 3.4 min. During the test, expired air was collected in a series of meteorologic balloons. After the test, the percentages of O_2 and CO_2 in the expired air were determined by the electronic gas analyzers. The volume of expired air in each balloon was measured by using a Tissot gasometer.

The peak oxygen deficit was determined by the method developed by Hermansen and Medbø (27) and described in detail by Medbø et al. (33). Peak oxygen deficit was calculated as the difference between the total oxygen uptake (liters) and the estimated total energy required during the supramaximal bout of exercise. The total energy required was calculated as the product of the rate of energy expenditure and the exercise duration. The rate of energy expenditure during supramaximal running was estimated by extrapolation of the linear relationship between $\dot{V}O_2$ and treadmill speed at submaximal running intensities measured in the first laboratory session (27). During horizontal running, the mean slope and intercept for the equation predicting $\dot{V}O_2$ (l/min) from speed (km/h) were 0.19 ± 0.05 (SD) and 0.24 ± 0.35 (r = 0.99 ± 0.08). During uphill running, the corresponding slope and intercept were 0.29 ± 0.07 and 0.35 ± 0.37 (r = 0.99 ± 0.13). Anaerobic capacity was estimated from peak oxygen deficit by multiplying by 0.9 to correct for oxygen stores (33, 39). The single-trial reliability [intraclass correlation from one-way analysis of variance (ANOVA) (11)] of peak oxygen deficit, determined in a subsample of nine subjects who were tested twice under one of the conditions (horizontal or uphill) was 0.85. The means for *trials 1* and *2* were 2.882 and 2.774 l/min, and the within-subjects SD of replicate measures was 0.37 l/ min.

The third test session under each condition involved collection of data needed to assess the right lower extremity muscle activation during running from exercise-induced contrast shifts in proton-weighted MR images. MR images were obtained by using a 1.5-T superconducting magnet (General Electric, Milwaukee, WI) at rest before exercise and immediately after the supramaximal running. Before the first image-collection sequence, the subject remained inactive for 10 min. After the rest period, preexercise MR images were obtained of the subject's right lower extremity. The scanning procedure and data analysis were similar to that described previously (2, 38). Before image collection, an external landmark was placed on the thigh, 20 cm distal to the iliac crest. The exact location of the external landmark was recorded on acetate paper. The acetate paper was reapplied at the next test session to ensure that the placement of the landmark in the magnet bore was consistent for each test session. The external landmark was aligned with the crosshairs of the imager before each scan. A resting scan was obtained of the region between the iliac crest and the patellar crest. A series of contiguous transaxial images (10 mm thick and spaced 20 mm apart) were obtained throughout the entire region. A second series of transaxial images 10 mm thick spaced 20 mm apart were obtained between the patellar crest and the ankle. Proton transverse relaxation time (T_2) weighted images (repetition time/echo time = 1, 300/30, 60 ms) were obtained within a 40-cm rectangular field for each image-collection sequence. A 256 × 256 matrix resolution and one excitation were used. Total scan time was 364 s, 182 s for each of the two successive scans (iliac crest to patellar crest and patellar crest to ankle).

The subject then completed a supramaximal (115% of $\dot{V}O_{2peak}$) bout of treadmill running to exhaustion. Mean treadmill time was 2.6 ± 0.5 min, and it ranged from 2.0 to 3.9 min. Immediately after exercise, MR images of the right lower extremity were again obtained following procedures described for resting conditions. The time from termination of exercise to initiation of postexercise image attainment varied between subjects but was held constant for the two conditions for each individual. The time from termination of exercise to completion of postexercise scans ranged from 10.9 to 12.3 min.

MR images were transferred to a Macintosh computer and analyzed by using a modified version of the public-domain National Institutes of Health image software (written by Wayne Rasband at NIH and available from the internet at http://zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Road, Springfield, VA 22161, part no. PB93-504868). For each image, regions of interest were defined by tracing each muscle or muscle group in the cross section. The 13 muscle regions of interest were iliopsoas, gluteus maximus-medius-minimus, sartorius, rectus femoris, vastus lateralis-medialis-intermedius, adductor magnus-longus-brevis, gracilis, biceps femoris, semitendinosus, semimembranosus, gastrocnemius, soleus, and tibialis anterior, plus all calf musculature except the soleus and gastrocnemius. After spatial calibration, muscle cross-sectional area and T_2 were determined for each region of interest. A T_2 (two-thirds of the signal decay time) was calculated for each pixel within a region of interest by using the formula $T_2 = (t_a - t_b)/\ln(i_a/i_b)$, where t_a and t_b are spin-echo collection times and i_a and i_b are signal levels at times a and b. In the preexercise scans, pixels with T_2 values between 20 and 35 ms were assumed to represent muscle at rest on the basis of reports in the literature that resting T_2 of muscle is $\sim$28 ms with a SD of 0.3–4 ms (2). Mean T_2 values for muscle in our subjects at rest were 29.7 ± 0.6 and 29.7 ± 0.4 ms before horizontal and uphill running, respectively. Pixels with T_2 values out of this range were considered to be nonmuscle. This nonmuscle cross-sectional area was later subtracted from the postexercise muscle images taken on that day.

In the postexercise scans, pixels with T_2 values greater than the resting mean + 1 SD were assumed to represent active muscle (muscle that had recently performed contractile activity). This criterion has been used previously (2, 15, 38) and, although arbitrary, is supported by observations that *1*) resting T_2 in the present study was similar to that in previous studies (2); *2*) only a small amount of low-intensity exercise is needed to significantly increase T_2 values (49); *3*) elevations in T_2 within the range observed in the present study are directly related to electromyogram activity, force, and rate of work (1, 19, 30); *4*) estimates of the cross-sectional area of muscle activated increase in direct proportion to force and exercise intensity (2, 38); and *5*) estimates of the percentage of individual muscles activated using this criterion are reasonable, ranging from $\sim$25% to >90% (2, 15, 37, 38). The active muscle and total muscle cross-sectional areas for each region of interest were determined.

Volumes of active and total muscle were determined by summing the products of the cross-sectional areas and the thickness of each section (10-mm thickness plus 20-mm space) for each region of interest (24). The volumes for the 13

regions of interest were then summed. The total active muscle volume was divided by the total postexercise muscle volume to obtain the percentage of total muscle volume that was active. Postexercise total muscle volumes were not different ($P > 0.05$) between horizontal and uphill conditions.

The single-trial reliability coefficients (intraclass correlation from 1-way ANOVA) for the volume of lower extremity muscle and T_2 at rest based on three determinations on separate days were 0.90 and 0.47, respectively. The within-subjects SDs of replicate measurements were 53 cm^3 and 0.49 ms. The reliability of T_2 was low because the range of values was extremely small (28.5–30.7 ms) on each occasion. There were no significant differences among the three means (29.7 ± 0.6, 29.7 ± 0.4, and 29.6 ± 0.5 ms). Three separate measures of the same scan of muscle cross-sectional area at rest were obtained for 30 individual muscles. On the basis of these data, the intraclass reliability coefficient for repeated determinations of a muscle cross-sectional area was 0.99. The within-subjects SD of replicate measurements was 0.53 cm^2.

At the final test session, a whole body scan was obtained for each subject by using dual-energy X-ray absorptiometry (DEXA) (Hologic QDR 1000-W, software version 5.5) to determine percent body fat.

Statistical analysis. A *t*-test for dependent samples was used to determine the significance of differences between variables measured during uphill and horizontal running. A significance level of $P \leq 0.05$ was used for each variable. The relationship between the differences in peak oxygen deficit and the differences between the right lower extremity active muscle volume between uphill and horizontal running was evaluated by using simple regression and correlation. Statistical analyses were conducted by using Systat for Windows, version 5 (SPSS).

RESULTS

The mean $\dot{V}O_{2peak}$ during uphill running (2.90 ± 0.50 l/min) was significantly greater ($P < 0.05$) than for horizontal running (2.82 ± 0.50 l/min) by 3%. The mean peak oxygen deficit measured during uphill running (2.96 ± 0.63 liters or 49 ± 6 ml/kg) was significantly greater ($P < 0.05$) than the mean peak oxygen deficit measured during horizontal running (2.45 ± 0.51 liters or 41 ± 7 ml/kg) by 21%. Individual values ranged from 1.97 to 3.89 liters and from 1.63 to 3.4 liters for uphill and horizontal conditions, respectively (Fig. 1).

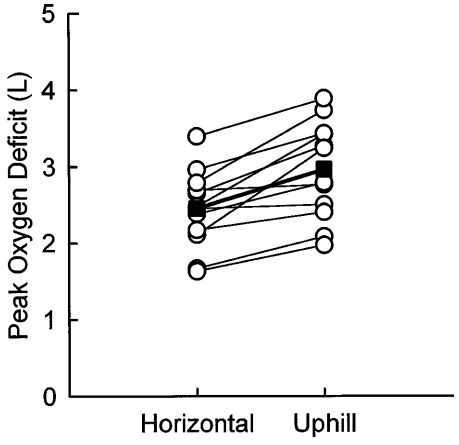

Fig. 1. Peak oxygen deficit for horizontal and uphill (10% grade) supramaximal treadmill running. Thick line connects mean values (■) for each condition.

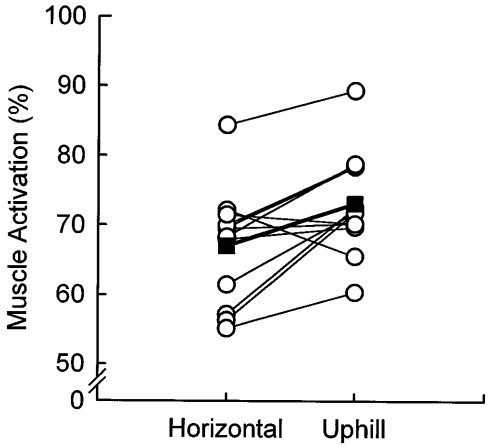

Fig. 2. Percentage of right lower extremity muscle volume activated for horizontal and uphill (10% grade) supramaximal treadmill running. Thick line connects mean values (■) for each condition.

Mean muscle volume for the right lower extremity at rest measured from MR images was 3,910 ± 678 (SD) cm^3. Right lower extremity muscle mass was estimated as the product of the volume and the density of muscle (1.056 g/cm^3) (35). Doubling this value indicated that the mean muscle mass for both lower extremities was 8.3 kg (range 5.7–12.4 kg).

The mean right lower extremity active muscle volume measured during uphill running (2,990 ± 824 cm^3) was significantly greater ($P < 0.05$) than that measured during horizontal running (2,726 ± 815 cm^3) by 10%. The active muscle mass for both lower extremities was 6.1 kg for uphill running and 5.6 kg for horizontal running. The mean percentage of muscle volume activated measured during uphill running (73.1 ± 7.4%) was significantly greater ($P < 0.05$) than that measured during horizontal running (67.0 ± 8.3%) by 9%. Individual values ranged from 60.4 to 89.3% and from 55.2 to 84.4% for uphill and horizontal conditions, respectively (Fig. 2).

The mean T_2 for the lower extremity muscle regions of interest was used to represent the intensity of muscle use. Mean T_2 during horizontal running (34.0 ± 0.9 ms) was not significantly different ($P > 0.05$) from that for uphill running (34.2 ± 1.0 ms). The relationship ($y = 8.05 \times 10^{-4}x + 0.35$; $r = 0.63$, SE of estimate = 0.29 liter) between the differences between uphill (10% grade) and horizontal treadmill running peak oxygen deficit (liters) and muscle volume activated (cm^3) (Fig. 3) was significantly different from zero ($P < 0.05$) and was moderately strong.

DISCUSSION

In recent years, exercise-induced contrast shifts in MR images have been used to determine the pattern and intensity of muscle activation during exercise (1, 2, 19, 20, 22, 23, 30, 38, 41, 46). We used this relatively new technology to determine whether the greater peak oxygen deficit during uphill compared with horizontal running was related to a greater active muscle volume or mass. The primary finding of this study was that the increase in peak oxygen deficit was related to an

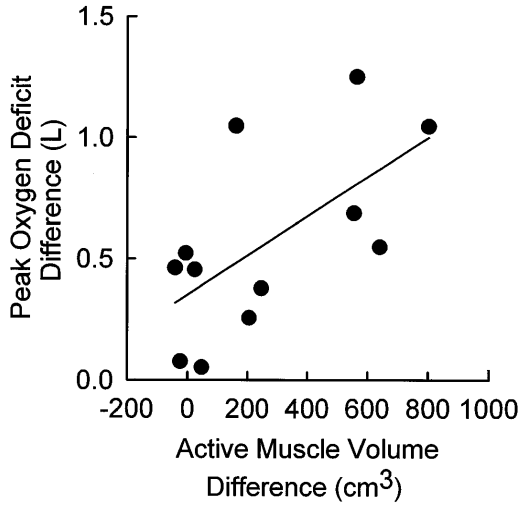

Fig. 3. Scatterplot of differences between uphill (10% grade) and horizontal treadmill running peak oxygen deficit and percentage of right lower extremity muscle activated. $y = 8.05 \times 10^{-4}x + 0.35$; $r = 0.63$, SE of estimate = 0.29 liter.

increase in lower extremity muscle volume activated during uphill compared with horizontal running. Because the intensity of muscle use as measured by the T_2 values was the same, this finding suggests that greater motor unit recruitment is in part responsible for higher peak oxygen deficit during uphill running. However, the moderate correlation ($r = 0.63$) between the increase in peak oxygen deficit and the increase in muscle activation indicates that other factors, such as increased efficiency, probably also contribute to the greater peak oxygen deficit measured during uphill compared with horizontal running.

Our findings support previous observations that the peak oxygen deficit determined during supramaximal treadmill running increases as a function of treadmill incline (36, 44). Olesen (36) determined the peak oxygen deficit during treadmill running at 1, 10, 15, and 20% grade. Compared with running at 1% grade, peak oxygen deficit increased 37% at 10.5% grade and ~80% at 15% grade, but it failed to increase further as incline was increased to 20% grade. Walker et al. (44) reported findings similar to those of Oleson (36). They found peak oxygen deficit increased 26% as treadmill grade was increased from 0 to 10%. In the present study, peak oxygen deficit increased 21% as treadmill incline was increased from 0 to 10% grade. The reason why the increase in our study was so much smaller than in Oleson's study is unclear. It may be related to the substantially higher state of aerobic training, as reflected by the much higher $\dot{V}O_{2peak}$ values, and to the fact that most of the subjects in her study were men with more muscle and less body fat, which may have resulted in a greater capacity to run uphill compared with horizontally.

The mechanism(s) responsible for the increase in peak oxygen deficit with an increase in treadmill incline is unknown but has been hypothesized to reflect increased active muscle mass and/or altered running efficiency (6, 9, 36, 44). An association between anaerobic capacity and active muscle volume or mass should exist (4, 40), because anaerobic energy is released within the active musculature and is not dependent on oxygen supply. Peak oxygen deficit has been shown to be directly related to the size of the musculature involved in different modes of exercise. Åstrand and Saltin (4), reported that peak oxygen deficit increased 60–70% when determined during whole body exercise (leg cycling + arm cranking), compared with leg cycling alone. Weyand et al. (47) reported that peak oxygen deficit measured during two-leg cycling was double that measured during one-leg cycling and that there was a strong association between peak oxygen deficit and estimated fat-free volume of active leg or legs. Bo et al. (12) reported that the peak oxygen deficit for a group of competitive oarsmen was significantly greater during rowing, which involves the upper and lower extremities, than during running, which involves primarily the lower extremities.

Walker et al. (44) found that peak oxygen deficit during supramaximal running at 10% grade while individuals carried added external weight equal to 15% body weight was 14% greater than that measured during running at 10% grade. The authors hypothesized that the greater peak oxygen deficit with added weight probably reflected increased active muscle mass because during submaximal running at the same speed, $\dot{V}O_2$ per unit weight carried (running economy) is not changed significantly or is slightly less with added weight compared with normal weight (17).

Our finding that the muscle volume activated is greater for uphill (10% grade) than for horizontal supramaximal treadmill running is consistent with that of Costill et al. (16), who reported glycogen depletion in the lower extremities during uphill (6% grade) compared with horizontal running at the same relative intensity. Three subjects each completed 2 h of treadmill running at 75% of maximal $\dot{V}O_2$ determined for each condition. Glycogen depletion in units of glucose in the vastus lateralis (55 mmol/kg), gastrocnemius (60 mmol/kg), and soleus (46 mmol/kg) muscles after uphill running was greater than after horizontal running (17, 44, and 33 mmol/kg, respectively).

Olesen (36) suggested that a difference in active muscle mass between uphill and horizontal supramaximal running could not account for the 82% difference in peak oxygen deficit that she observed between subjects who were running at 1 and 15% grades, on the basis of the assumption that a proportional increase in muscle activation would be required. Bangsbo (6) agreed that a difference in active muscle mass between uphill and horizontal supramaximal running could not account for the entire difference in peak oxygen deficit reported by Oleson (36). He suggested that if 15 kg of active muscle mass are assumed for running at 1% grade, then it follows that 27 kg are active for running at 15% grade to account for the difference in peak oxygen deficit reported by Oleson (36) (82% higher at 15 than at 1% grade). In reply, Medbø (6) pointed out that 15 kg of active muscle mass during running at 1% grade are merely an estimate and may be exaggerated. In addi-

tion, it is unknown whether a proportional increase in muscle activation would be needed to account for the increase in peak oxygen deficit observed.

We found that a mean increase in the peak oxygen deficit of 0.51 liter (21%) during uphill compared with horizontal running was associated with a 264-cm^3 (9%) mean increase in active muscle volume and a 0.5-kg (9%) mean increase in active muscle mass. These percentages indicate that the increase in the peak oxygen deficit does not increase in proportion to the increase in active muscle mass during running, as has been assumed (6, 36). The correlation between the increase in the peak oxygen deficit and the increase in active muscle volume was only moderately strong (r = 0.63), indicating that the increase in active muscle volume accounted for ~40% of the variance in peak oxygen deficit. The moderate relationship between peak oxygen deficit and active muscle volume could reflect the measurement error associated with both techniques utilized (determination of peak oxygen deficit and active muscle volume) and/or the fact that the contribution of upper-extremity muscle activation to the peak oxygen deficit was not evaluated.

Another possibility is that the increase in peak oxygen deficit with treadmill incline is explained by multiple mechanisms. It is possible that the increase in peak oxygen deficit is due to an increase in active muscle volume or mass as well as to an increase in the intensity of muscle use. The present data do not support this notion; mean T_2 values, reflecting the intensity of muscle use, were not different between horizontal and uphill running. Olesen (36) suggested that the increase in peak oxygen deficit may be due to decreased running efficiency associated with uphill running and an underestimation of peak oxygen deficit during horizontal running. Although efficiency defined as the ratio of work done to energy cost has been estimated for horizontal running (14), we are not aware of comparisons between horizontal and uphill conditions. Decreased efficiency during uphill running could reflect a decreased use of stored elastic energy (3) and an increase in the ratio of positive to negative work (5). A definitive answer to this question depends on development of a valid method for determining total work done during horizontal and uphill running.

The validity of using the peak oxygen deficit to assess anaerobic capacity is controversial (7, 32). The primary criticism of the measure is related to the assumption that the energy demand during supramaximal intensities of exercise can be estimated by extrapolation of the relation of $\dot{V}O_2$ to exercise intensity during submaximal exercise. The accuracy of this assumption cannot be adequately determined without a definitive measure of energy demand during high-intensity exercise. The validity of the method is supported by studies that have found estimates of anaerobic energy release from metabolite changes in active skeletal muscle and from the peak oxygen deficit during one-leg knee-extension exercise (8) and two-leg cycling (48) to be quantitatively similar and highly correlated (34) in untrained subjects. Green et al. (26), however, found the peak oxygen deficit was unrelated to anaerobic energy release estimated from muscle metabolite changes in highly trained cyclists. Disparity between these studies may be related to the muscle mass involved in the exercise (small vs. large), the level of training of the subjects, and/or the conditions under which the data were collected (one intensity vs. several intensities), which affects the variability of the data and the strength of relationships reported.

Estimates of anaerobic capacity from metabolite changes in muscle and from measures of peak oxygen deficit have been compared by expressing both in millimoles ATP per kilogram active muscle. Knowledge of the active muscle mass is crucial to a valid comparison. In the studies cited above, active muscle mass was estimated from thigh volume estimated by anthropometric measures (8) or as 25% body mass (26, 34, 48). The latter approach has also been used in discussion of the active muscle mass during running (33), but it does not appear to be based on any direct measures. Estimates of active muscle mass during cycling or running from body mass are crude at best and do not take into account differences in body fatness, particularly between men and women. Furthermore, none of the approaches for estimating active muscle mass during exercise has taken into account variation in the percentage of available muscle that is actually activated. Bangsbo (6) and Medbø (32) have pointed out that evaluation of the validity of the peak oxygen deficit based on comparisons with anaerobic energy release estimated from muscle metabolite changes is limited by lack of knowledge of the active muscle mass during large-muscle activity.

On the basis of the assumption that the active muscle mass was 25% body mass during two-leg cycling, Medbø and Tabata (34) and Withers et al. (48) found that the anaerobic capacity in men with a mass of 70–75 kg estimated from metabolite changes in muscle was ~60 mmol ATP/kg active muscle. These values were very similar to anaerobic capacities estimated from the peak oxygen deficit, assuming 1 mol O_2 = 6.5 mol ATP. If it is assumed that men in these studies were 12% fat, then the assumed active muscle was 28% of the fat-free mass. By taking into account that women in the present study averaged 20% fat, and assuming muscle was the same fraction of the fat-free mass as for men, then the active muscle mass would be estimated to be 13.5 kg and the anaerobic capacity 63.5 mmol ATP per kilogram active muscle. Thus, by using the same approach for estimating active muscle mass, our estimates for anaerobic capacity per unit active muscle are similar to previous estimates. However, the estimates of active muscle mass based on the MR imaging in the present study, 5.6 kg during horizontal running and 6.1 kg for uphill running, are less than one-half of those assumed by the estimation from body mass. This means that the anaerobic capacities per unit active muscle mass during horizontal and uphill running are calculated to be much higher (127 and 141 mmol ATP/kg). These values are considerably higher than previous estimates of the anaerobic capacity expressed

per unit active muscle (8, 34, 48). There are two possible explanations: *1*) our estimates of the active muscle mass are too low or *2*) previous estimates of the active muscle mass are too high. Both may be correct.

In the present study, mean muscle mass for both lower extremities was 8.3 kg (SD) (range 5.7–12.4 kg). This is lower than comparable estimates for women of approximately the same size from anthropometry (42) and from dual-photon absorptiometry or DEXA (25, 29), which estimate lower extremity muscle in young women at ~11 kg. However, anthropometric estimates that assume the limb is a circle overestimate limb muscle cross-sectional areas (10, 13, 28) and estimates of appendicular muscle from DEXA overestimate values from computerized-tomography (CT) scanning, because other nonfat soft tissues are included in the estimates from DEXA (45). Our cross-sectional muscle areas measured by MR imaging may be somewhat lower than those from CT scanning because the area of pixels with T_2 values outside the range for resting muscle is subtracted from the traced muscle regions of interest. Therefore, our somewhat low estimates appear to be consistent with the method used. Estimates of muscle cross-sectional areas or volumes from MR imaging and CT scanning are generally considered to be the most accurate of the noninvasive approaches (45), and we have no reason to believe that our measures are not valid. We acknowledge that the measure of the lower extremity muscle does not include all of the musculature that is active during running; trunk and arm muscles are also used but to a lesser extent. This activity was not measured in the present study and is a limitation.

One of the principal findings of the study was that not all of the muscle in the lower extremity is activated during strenuous horizontal and uphill running. On the basis of the changes in T_2 values from MR scans, only ~67% of the lower extremity muscle was activated during exhaustive horizontal running, and only ~73% of the lower extremity musculature was activated during uphill running at 10% grade. To our knowledge, these are the first data available on the percentage of muscle in the lower extremity that is activated during running. The finding that only ~70% of muscle in the lower extremity is activated may reflect the ballistic, dynamic nature of movements during running and the fact that near-maximum levels of force are not needed. In addition, in a 2- to 3-min effort, the maximal rate of power output is less than during shorter efforts (34), which should result in less muscle mass activated, assuming muscle activation is proportional to power output. In large-muscle activity, motor units rely primarily on recruitment and less on rate coding to modulate force (18). Studies have shown that the increased signal intensity and the estimated percentage of muscle activated in proton-weighted MR images are directly related to the rate of work done (power output) across the range of submaximal and maximal intensities (1, 2, 19, 30, 38).

The less than complete activation of all available muscle is consistent with other studies using the same approach with other modes of exercise in which relatively high levels of force are developed. For example, Adams et al. (2), using the same MR imaging methodology, estimated that, with electromyostimulation, 100% maximal voluntary isometric torque during 5 sets of 10 muscle actions could be achieved by activating ~71% of the quadriceps femoris. Ploutz et al. (38) found that 74–83% of the quadriceps femoris was activated during 5 sets of 10 knee extensions at 100% of maximal load. Ploutz and Dudley (37) found that 6 sets of 10 repetitions to exhaustion of the squat exercise activated 91% of the vasti, 68% of the rectus femoris, and 66% of the adductor muscle groups. And, Conley et al. (15) found that 5 sets of 10 neck movements to exhaustion resulted in activation of from 0 to 90% of the cross-sectional area of various individual muscles in the neck. In the present study, we found that the percentage activation of individual muscles or muscle groups in the lower extremity ranged from 41 to 90% during horizontal running and from 44 to 83% during uphill running. The findings of these studies indicate that involved muscles are not uniformly or maximally activated during strenuous exercise. The studies involving heavy-resistance exercise are consistent with the concept that there may be a neural limitation to motor unit recruitment in some forms of exercise that prevents the full force potential of muscle from being utilized during voluntary contractions (18). This concept may apply to exhaustive running as well if power output is limited by the muscle mass recruited.

It is possible that the method used to determine active muscle in the present study may underestimate the muscle actually involved in contractile activity. First, in voluntary muscle contractions s uch as running, it is possible that motor units may be intermittently activated but fail to reach the threshold needed to alter T_2 sufficiently to be considered active. This effect is apparent in the study of Adams et al. (2), in which voluntary contractions at 25 and 50% of maximal voluntary isometric torque resulted in minimal T_2 changes, whereas the same levels of torque elicited via electrical stimulation elicited clear elevations, apparently because of lack of motor unit cycling. Second, the criterion used to designate T_2 values as active (T_2 increase greater than the resting mean $\pm$ 1 SD) in this and previous publications using the same method (2, 38) is fairly conservative and might reject pixels that represent active mucle regions. Third, because two scans were needed to assess muscle activation in the upper and lower leg, the time after exercise until the scans were completed was longer than usual. This resulted in T_2 values for pixels in the lower leg to be somewhat lower than if two scans had not been required. However, the mean T_2 after horizontal and uphill running was ~3 SDs above the criterion, indicating that there would be few pixels that initially exceeded the criterion, that were not counted as active because the T_2 had fallen below the criterion during the first 10–12 min of recovery. Because the range of values for estimates of the percentage of muscle voluntarily activated in this and other studies using this method

(15, 37) extends to over 90% in individual muscles, underestimation, if any, would appear to be small. Even if the absolute values for estimated active muscle mass are somewhat low because of a bias in the method, the bias should be same for uphill and horizontal running and should not affect our finding of greater relative muscle activation during uphill compared with horizonal running.

The exact cellular mechanisms underlying the exercise-induced contrast shifts in T_2 of MR images are unknown. Because proton-weighted MR images are based on signals from hydrogen atoms and because the primary source of hydrogen in the human body is water, exercise-induced changes in muscle T_2 are believed to be caused by movement of water into and among compartments in muscle. However, simple movement of water into muscle does not fully explain T_2 changes with exercise, because increased muscle cross-sectional area due to venous occlusion is not associated with increased T_2 (19). Increased muscle perfusion also is an unlikely cause, because muscle T_2 increases after exercise with vascular occlusion (21). Patients with McArdle's disease, who lack phosphorylase, the enzyme needed for breakdown of muscle glycogen, and who do not experience lactate accumulation during heavy exercise, do not have T_2 increases after exercise (22). This finding suggests that glycogenolysis and/or lactate accumulation is responsible for exercise-induced T_2 changes. Studies showing that T_2 is negatively correlated with pH and the inorganic phosphate-to-phosphocreatine ratio support this suggestion (46). Thus the biochemical events associated with muscle recruitment and the consequent increased energy demand appear to be responsible for the exercise-induced contrast shift in MR images. Greater reported lactate accumulation (36) accompanying greater muscle activation during exhaustive uphill running at 10% grade compared with exhaustive horizontal running is consistent with this mechanism.

Because estimates of the active muscle mass in previous studies estimating the anaerobic capacity per unit active muscle were not based on direct measures of available involved muscle and did not measure the muscle actually activated, they could be overestimates. If this is the case, it means that the anaerobic capacity of human muscle is higher than previously thought. Additional studies of the anaerobic capacity involving direct measures of the active muscle mass are needed to confirm this possibility.

The results of the present study provide new insight into the mechanism(s) responsible for the increase in peak oxygen deficit with increasing treadmill incline. We conclude that the higher peak oxygen deficit during uphill compared with horizontal running is due in part to increased activation of skeletal muscle in the lower extremity. Additional research is needed to verify whether other potential mechanisms, such as altered running efficiency, contribute to this phenomenon.

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