Glutamate availability is important in intramuscular amino acid metabolism and TCA cycle intermediates but does not affect peak oxidative metabolism

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Glutamate availability is important in intramuscular amino acid metabolism and TCA cycle intermediates but does not affect peak oxidative metabolism. J Appl Physiol 105: 547–554, 2008. First published May 29, 2008; doi:10.1152/japplphysiol.90394.2008.—Muscle glutamate is central to reactions producing 2-oxoglutarate, a tricarboxylic acid (TCA) cycle intermediate that essentially expands the TCA cycle intermediate pool during exercise. Paradoxically, muscle glutamate drops ~40–80% with the onset of exercise and 2-oxoglutarate declines in early exercise. To investigate the physiological relationship between glutamate, oxidative metabolism, and TCA cycle intermediates (i.e., fumarate, malate, 2-oxoglutarate), healthy subjects trained (T) the quadriceps of one thigh on the single-legged knee extensor ergometer (1 h/day at 70% maximum workload for 5 days/wk), while their contralateral quadriceps remained untrained (UT). After 5 wk of training, peak oxygen consumption (V˙O2peak) in the T thigh was greater than that in the UT thigh (P < 0.05); V˙O2peak was not different between the T and UT thighs with glutamate infusion. Peak exercise under control conditions revealed a greater glutamate uptake in the T thigh compared with rest (7.3 ± 3.7 vs. 1.0 ± 0.1 μmol·min⁻¹·kg wet wt⁻¹, P < 0.05) without increase in TCA cycle intermediates. In the UT thigh, peak exercise (vs. rest) induced an increase in fumarate (0.33 ± 0.07 vs. 0.02 ± 0.01 mmol/kg dry wt, P < 0.05) and malate (2.2 ± 0.4 vs. 0.5 ± 0.3 mmol/kg dw, P < 0.05) and a decrease in 2-oxoglutarate (12.2 ± 1.6 vs. 32.4 ± 6.8 μmol/kg dw, P < 0.05). Overall, glutamate infusion increased arterial glutamate (P < 0.05) and maintained this increase. Glutamate infusion coincided with elevated fumarate and malate (P < 0.05) and decreased 2-oxoglutarate (P < 0.05) at peak exercise relative to rest in the T thigh; there were no further changes in the UT thigh. Although glutamate may have a role in the expansion of the TCA cycle, glutamate and TCA cycle intermediates do not directly affect V˙O2peak in either trained or untrained muscle.

alanine; glutamine; exercise training; ammonia

EXPANSION OF TRICARBOXYLIC ACID (TCA) cycle intermediates (anaplerosis) has been known to occur with the onset of exercise (7, 10, 19, 26). TCA cycle anaplerosis is generally explained by an increased flux through the near-equilibrium reaction of alanine aminotransferase (AAT), generating alanine and 2-oxoglutarate from glutamate and pyruvate, where the production of 2-oxoglutarate essentially expands the TCA cycle intermediate pool. Elevated flux through this reaction is attributed to a transiently greater pyruvate production relative to its rate of oxidation in the pyruvate dehydrogenase (PDH) reaction (10–12, 14). However, the physiological importance of TCA cycle anaplerosis remains unclear. It has been purported that anaplerosis may be necessary to achieve adequate energy provision (26, 30, 31). Several studies have tested this theory but have not found an association between TCA cycle anaplerosis and oxidative metabolism at variable relative intensities of submaximal exercise (7, 8, 19). If anaplerosis drives the flux of the TCA cycle, then the expansion of the TCA cycle intermediate pool would also be a determinant of peak oxidative capacity. To date, TCA cycle anaplerosis has not been investigated during peak oxygen consumption (V˙O2peak).

While the AAT reaction may be responsible for the initial rise in 2-oxoglutarate, this particular TCA cycle intermediate is the first to decline after the onset of exercise (10) and achieves the greatest drop relative to other intermediates during prolonged exercise (14). This phenomenon has led to the hypothesis that 2-oxoglutarate, which is the substrate of a flux-generating step in the TCA cycle, may be associated with fatigue (26, 30, 31). Concomitantly, muscle glutamate exhibits a 40–80% drop with the onset of exercise despite the 10-fold increase in uptake from the circulation (8, 10). Since glutamate is central to several near-equilibrium reactions that interlink with the TCA cycle, the drop in muscle glutamate may have implications for the TCA cycle intermediate pool, which would ultimately limit the AAT reaction and induce a decline in 2-oxoglutarate. Oral or intravenous glutamate supplementation can directly or indirectly enhance muscle glutamate uptake (25, 27–29). Greater glutamate availability may increase the mass-action effect of the glutamate-based transamination reactions, resulting in greater 2-oxoglutarate production. While glutamate supplementation has been used in a limited number of exercise protocols in healthy subjects (25) and patients with heart disease (27–29), the exercise-induced relationships between intramuscular amino acids and TCA cycle intermediates have not been evaluated.

We hypothesized that if peak oxidative capacity is related to an increase in TCA cycle intermediates, training would result in a greater V˙O2peak concurrently with a rise in TCA cycle intermediates. If muscle glutamate is limiting the flux through the AAT reaction, then increasing glutamate availability would increase flux through AAT and result in further anaplerosis of the TCA cycle for an increase in oxidative capacity. Intravenous glutamate supplementation would increase glutamate availability to test the role of glutamate in relation to the TCA cycle as well in intermediary metabolism.

METHODS

Seven healthy, recreationally active male subjects were recruited to participate in this study. The mean ± SE age, body mass, and height...
of the subjects were 23 ± 1 yr, 84.7 ± 8.9 kg, and 184.7 ± 3.8 cm, respectively. Subjects were informed, verbally and in writing, of the purpose of the study as well as the procedures and risks involved with the experiment. The experimental protocol was approved by the Copenhagen and Frederiksberg Ethics Committee.

**Experimental protocol.** Subjects underwent supervised training sessions using the one-legged knee extensor model, which isolated exercise to the quadriceps muscles. The training protocol consisted of 1-h training of one thigh at ~70% of the predetermined peak work rate for five sessions per week for 5 wk. The contralateral thigh was not exposed to any exercise for the duration of the training protocol. Selection of the training thigh was randomized to avoid potential effects of dominance. After completion of the training sessions, subjects were provided with a standard mixed diet that matched their individual daily caloric intake. All subjects completed the training protocol. Two days subsequent to the completion of the training period, subjects arrived at the laboratory after an overnight fast to undergo the testing protocol. Catheters were inserted under local anesthesia in the femoral artery of one leg and the femoral vein of the other leg. The femoral artery was cannulated at ~2 cm below the inguinal ligament, set proximally ~10 cm, and was connected to a blood pressure transducer and monitor. The femoral venous catheter was placed ~2 cm below the inguinal ligament and forwarded distally ~10 cm. Thermistors were placed in each femoral vein for blood flow measurements. After the catheters were inserted, subjects rested 30 min before resting blood samples were drawn and resting muscle biopsies were taken from both thighs. Muscle biopsies were obtained from the vastus lateralis muscle under local anesthesia with the needle biopsy technique as described by Bergström (4).

Peak work rates were attained during the knee extensor exercise by incrementally increasing the work rates every 2 min by a predetermined amount that was based on the results of the peak tests performed before training was initiated. Once the exercise was completed in one thigh, there was 15 min of rest before the contralateral thigh was tested; the order in which incremental exercise was conducted was between the untrained (UT) and trained (T) thighs was randomized. During testing, subjects were encouraged to increase their work rates to achieve the greatest work rate possible for each thigh. Cessation of exercise occurred with voluntary exhaustion. Blood samples and blood flow measurements were taken at the same time points for each thigh. Femoral venous blood flow measurements were made with the thermodilution technique (1), which is largely representative of the quadriceps blood flow during the knee extensor exercise. To avoid contamination of blood flow from the lower leg, an occlusion cuff was placed just below the knee and inflated to >240 mmHg for 30 s before cold saline was infused through the thermistor. Thigh blood flows were taken every 40 and 90 s after a change in work rate and were calculated with the heat balance equation. Blood samples were taken 60 s after the change of work rate; the blood and flow measurements taken at peak exercise are presented here. Muscle biopsies were taken from the exercising thigh immediately at the cessation of each exercise bout (Fig. 1).

This protocol was carried out first under control conditions, and subsequently a second experiment was conducted in which each thigh underwent a second peak exercise test (24) followed by a glutamate infusion trial. The second experiment was an affiliated study that was unlikely to impact on the glutamate trial since glutamate infusion commenced 45 min after the completion of the second experiment. This order of testing conditions was maintained to avoid any residual effects from the glutamate infusion on the control conditions. Alternative designs, such as testing on different days, would have been ethically inappropriate because of repeated catheterization and scientifically less suitable because of potential day-to-day variation in diet, hormone, and training status that may occur in the thigh. For the glutamate trial, subjects rested 45 min in the supine position before the infusion. A catheter was then inserted into the anecubital vein for the 30-min infusion of ~1 mg·kg body wt⁻¹·min⁻¹ of sterile glutamate dissolved in saline before the initiation of exercise. The infusion method was used to provide a steady supply of glutamate and to avoid the large, first-pass uptake of glutamate by the gut with oral supplementation. The infusion was continued during the exercise bout for each thigh as these bouts were approximately the same duration. With appropriate rest, short, consecutive, intense exercise bouts with the knee extensor muscles similar to the protocol here have not been found to induce an effect on energy metabolism (2). Although seven subjects had completed the training protocols, because of the technical challenges and invasiveness of this study, only five subjects completed the testing protocol (n = 5 for all data presented). Despite this sample size, the unique design and precise techniques provided essential groundwork demonstrating the usefulness of glutamate supplementation in future investigations.

**Blood and muscle analyses.** Heparinized syringes were used to collect blood samples for measuring blood PCO₂ and PO₂ (ABL5, Radiometer), hemoglobin, oxyhemoglobin fraction (OSM3 hemoximeter, Radiometer), and hematocrit. Hematocrit was measured in triplicate after microcentrifugation. Arterial and venous plasma samples from the heparinized syringes were used to analyze amino acids with HPLC (18), ammonia with a fluorometric method (3), and glucose and lactate with an automatic spectrophotometer (Cobas Fara, Roche).

Muscle biopsy samples were frozen immediately (<10 s) in liquid nitrogen. Biopsy samples were freeze-dried, powdered, and stored at −80°C. A portion of the freeze-dried muscle (~2.0 mg) was homogenized for 1 min in 100 μl of deionized water (Milli Q, Millipore, Bedford, MA) and then centrifuged for 3 min. The supernatant was analyzed for amino acids with HPLC (18). The remaining freeze-dried muscle was extracted with 0.5 M perchloric acid (with 1 mM EDTA) and neutralized with 2.2 M KHCO₃. The extract was then used to fluorometrically measure 2-oxoglutarate, fumarate, malate, lactate, and phosphocreatine (23). Of the TCA cycle intermediates, only 2-oxoglutarate, fumarate, and malate were measured. Fumarate and malate are TCA cycle intermediates that show the earliest increases and comprise >50% of the total TCA cycle intermediate pool (10).

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**Fig. 1.** The overall study design is illustrated for the data presented.
while 2-oxoglutarate is the only TCA cycle intermediate to decline early in exercise.

Muscle mass measurements. Magnetic resonance images (MRI) were obtained from the patella to the anterior inferior iliac spine after training. Twenty-eight scans were taken, and each scan was 3 mm thick with a distance of 17.1 mm between each scan. To calculate the muscle volume of the quadriceps the area of each scan was multiplied by the pixel area and then multiplied by the distance between sections. These scans were then summed to attain the total volume and were multiplied by 1.04 kg/L, which is assumed to be the density of muscle tissue. These muscle mass data were used to calculate VO2peak and flux of metabolites relative to the quadriceps muscle for each thigh.

Calculations and statistics. Values are expressed as means ± SE. Branched-chain amino acids (BCAA) represent the sum of valine, leucine, and isoleucine. All flux calculations were based on the Fick principle and blood flow measurements. To depict the relative rates and dynamic changes of amino acids and ammonia, total uptake/release of amino acids and ammonia was represented by compiling the flux of alanine, glutamine, glutamate, BCAA, and ammonia at rest and peak exercise for control and glutamate infusion conditions for each thigh. Plasma flows were calculated with hematocrit data. With a primary focus on metabolic changes during exercise, resting blood flow was only measured in one subject (0.3 l/min), and we used this value as the basis for flux calculations of all metabolites. In previous studies, resting blood flow is a consistent measurement of ∼0.3 ± 0.03 l/min (5, 10, 11). To identify an estimated balance of gluconeogenesis compared with insulin action, glucagon-to-insulin molar ratios were calculated by dividing the glucagon concentration by 3.485 and then by the insulin concentrations (20).

A series of paired Student’s t-tests were performed to test between UT and T muscle mass and time to reach peak exercise. Independent two-way repeated-measure (RM) ANOVAs were used to control for at least one variable and increase the power of the statistical analysis. The two-way RM ANOVAs independently tested the following: 1) training status (UT, T) versus activity (rest, peak exercise), 2) training status (UT, T) versus condition (control, glutamate), and 3) activity (rest, peak exercise) versus condition (control, glutamate). While three-way RM ANOVA would be appropriate, the sample size limited the use of this form of statistical analysis. Analysis was conducted with Tukey’s post hoc test. Statistical significance was accepted at P ≤ 0.05.

RESULTS

Muscle mass, work rate, and cardiovascular responses. After 5 wk of endurance training with the one-legged knee extensor model, muscle mass in the T quadriceps was greater by ∼0.2 kg (7%) compared with the UT thigh (3.09 ± 0.21 vs. 2.89 ± 0.21 kg, respectively; P < 0.05). During the control trial, peak work rate and VO2peak were ∼22% greater in the T thigh compared with the UT thigh (peak work rate: 101 ± 10 vs. 80 ± 7 W, P < 0.05; VO2peak: 1.42 ± 0.17 vs. 1.15 ± 0.15 l/min, P < 0.05; Table 1). VO2peak relative to quadriceps muscle mass also displayed a comparable difference between the T and UT thighs (0.44 ± 0.05 vs. 0.38 ± 0.05 l·min⁻¹·kg muscle⁻¹, respectively; P < 0.05), suggesting a greater oxidative capacity per unit of muscle mass in the T thigh. Peak work rate and VO2peak were achieved in 10.8 ± 0.9 min for the UT thigh and in 12.6 ± 1.0 min for the T thigh (P = not significant; Table 1). With glutamate infusion, peak work rates remained similar to control values for each thigh (T = 97 ± 8 W vs. UT = 79 ± 7 W; P < 0.05). Contrary to our hypothesis that glutamate infusion would elevate VO2peak, there were no statistically significant differences between control and glutamate trials in either thigh.

Table 1. Oxygen parameters in untrained and trained thighs during control and glutamate conditions

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<th>UT Thighs</th>
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<tr>
<td></td>
<td>Control</td>
<td>Glutamate</td>
</tr>
<tr>
<td></td>
<td>work peak</td>
<td>work peak</td>
</tr>
<tr>
<td>Work rate, W</td>
<td>80±7</td>
<td>79±7</td>
</tr>
<tr>
<td>Thigh blood flow, l/min</td>
<td>7.23±0.76</td>
<td>7.40±0.76</td>
</tr>
<tr>
<td>VO2, l/min</td>
<td>1.15±0.15</td>
<td>1.02±0.06</td>
</tr>
<tr>
<td>VO2, l·min⁻¹·kg⁻¹</td>
<td>0.38±0.05</td>
<td>0.35±0.03</td>
</tr>
<tr>
<td>Time to peak, min</td>
<td>10.8±0.9</td>
<td>10.0±1.4</td>
</tr>
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</table>

Data are means ± SE. T, trained; UT, untrained; VO2, oxygen consumption. *Significant difference (P < 0.05) between thighs under same conditions (control or glutamate infusion).

Changes in amino acids and ammonia in relation to training and glutamate infusion. Although all amino acids were measured in plasma and muscle, only key amino acids that relate to muscle glutamate metabolism are presented here. During peak exercise with the T thigh, arterial glutamate concentration decreased (rest 27.1 ± 2.4 μM vs. peak exercise 20.1 ± 2.0 μM; P < 0.05), implying that the T thigh may potentially be limited by the availability of circulating glutamate. Despite the lower arterial glutamate concentrations, glutamate uptake and alanine release were elevated with exercise in the T thigh compared with resting conditions (glutamate: 1.0 ± 0.1 vs. 7.3 ± 3.7 μmol·min⁻¹·kg wet wt⁻¹ at rest and peak exercise, P < 0.05; alanine: -1.6 ± 0.3 vs. -25.3 ± 9.0 μmol·min⁻¹·kg wet wt⁻¹ at rest and peak exercise, P < 0.05; Table 2). Muscle glutamate exhibited a -40% and 48% drop from rest to peak exercise in the T and UT thighs, respectively (Fig. 2), while other muscle amino acids did not change with peak exercise in either thigh. Unlike the T thigh, a greater release of glutamine and ammonia was demonstrated in the UT thigh during peak exercise compared with rest (glutamine: -20.3 ± 7.0 vs. -1.6 ± 0.6 μmol·min⁻¹·kg wet wt⁻¹, P < 0.05; ammonia: -74.9 ± 30.4 vs. 1.3 ± 0.6 μmol·min⁻¹·kg wet wt⁻¹, P < 0.05 (Table 2)).

Under control conditions, training appeared to facilitate nitrogen retention, particularly through an attenuated release of ammonia (Fig. 3) ; however, glutamate infusion appeared to induce an overall nitrogen loss that was similar to that in the UT thigh under control conditions.

With glutamate infusion, arterial glutamate concentrations and glutamate uptake were significantly elevated at rest compared with control for both thighs (Table 2). Over the 30-min infusion period at rest, this amounted to a total glutamate uptake of 233.8 ± 89.0 μM in the UT thigh and 872.6 ± 465.3 μM in the T thigh compared with 99.0 ± 8.5 and 92.4 ± 5.4 μM in the UT and T thighs, respectively, under control conditions for the same duration. Despite increased glutamate uptake at rest, muscle glutamate concentrations still dropped to the same extent as under control conditions (Fig. 2). At peak work rate for the T thigh, arterial glutamate tended to be lower than rest (P = 0.06) and was -50% lower than UT at peak exercise (P < 0.05, Table 2). The infusion of glutamate also coincided with significantly lower arterial ammonia concentrations during peak exercise of the T thigh compared with rest and compared with peak exercise under control conditions (Table 2). These data suggest that, with increased glutamate availability, there was increased clearance of glutamate, and
Table 2. Arterial concentrations and net uptake/release for amino acids, ammonia, lactate, and glucose at rest and peak work rate for each thigh under control and glutamate conditions

<table>
<thead>
<tr>
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<th>UT Thighs</th>
<th>T Thighs</th>
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<tr>
<td></td>
<td>Control</td>
<td>Glutamate</td>
</tr>
<tr>
<td></td>
<td>Rest Peak work rate</td>
<td>Rest Peak work rate</td>
</tr>
<tr>
<td>Arterial concentrations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>27.1±2.4 22.3±2.0</td>
<td>118.1±28.7†</td>
</tr>
<tr>
<td>Ala</td>
<td>186.0±25.8 230.1±29.8</td>
<td>136.6±14.8</td>
</tr>
<tr>
<td>Gln</td>
<td>292.7±38.4 301.0±43.7</td>
<td>276.7±49.1</td>
</tr>
<tr>
<td>Asp</td>
<td>1.2±0.1 1.1±0.1</td>
<td>2.0±0.4</td>
</tr>
<tr>
<td>BCAA</td>
<td>197.7±15.9 183.8±19.6</td>
<td>132.5±13.3†</td>
</tr>
<tr>
<td>Ammonia</td>
<td>34.9±4.9 63.4±18.4</td>
<td>32.6±8.3</td>
</tr>
<tr>
<td>Lactate</td>
<td>1.3±0.1 7.5±0.7†</td>
<td>1.8±0.4</td>
</tr>
<tr>
<td>Glucose</td>
<td>5.8±0.2 5.3±0.1</td>
<td>5.2±0.1</td>
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Net uptake/release

|                     |                                 |                                 |                                 |                                 |
| Glu                 | 1.1±0.1 5.6±2.0                 | 3.0±0.8†                        | 25.2±11.0†                      | 1.0±0.1                         |
| Ala                 | −1.3±0.2 −16.4±8.9              | −2.2±0.1                        | −24.3±15.5§                     | −1.6±0.3                         |
| Gln                 | −1.6±0.6 −20.3±7.0†             | −1.3±0.5                        | −36.7±18.2                       | −1.2±0.5                         |
| Asp                 | 0.02±0.01 −0.1±0.3              | 0.01±0.02                       | −0.6±0.05                       | 0.01±0.01                       |
| BCAA                | 0.7±0.3 4.7±4.2                 | −0.1±0.3                        | −1.3±3.6                         | 0.8±0.2                         |
| Ammonia             | 1.3±0.6 −74.9±30.4†             | 1.2±1.4                         | −38.7±15.8                       | 0.4±0.3                         |
| Lactate             | 0.00±0.0 −4.2±0.7†              | −0.4±0.1†                       | −9.6±1.7§                       | −0.0±0.01†                      |
| Glucose             | 0.04±0.01 0.4±0.1†              | 0.04±0.01                       | 0.5±0.2§                        | 0.04±0.01                       |

Data are means ± SE; negative numbers reflect release from the thigh and positive numbers reflect uptake. Arterial and flux data for amino acids and ammonia are expressed in μM and μmol·min⁻¹·kg wet wt⁻¹, respectively, while lactate and glucose are expressed in mM and mmol·min⁻¹·kg wet wt⁻¹, respectively. BCAA, branched-chain amino acids. *Significant difference (P < 0.05) between UT and T thighs at same time point and same conditions. Statistical differences within same thigh (P < 0.05); †vs. control rest, ‡vs. control peak work rate, §vs. glutamate rest.

Possibly ammonia, by other tissues during peak exercise of the T thigh.

With glutamate infusion at peak exercise, glutamate uptake was only greater in the UT thigh compared with the control trial (P < 0.05, Table 2). These conditions likely resulted in the greater alanine release and muscle glutamine concentrations at peak exercise in the UT thigh compared with control [alanine release: −16.4 ± 8.9 vs. −24.3 ± 15.5 μmol·min⁻¹·kg wet wt⁻¹, P < 0.05 (Table 2); muscle glutamine: 55.5 ± 8.8 vs. 40.5 ± 3.1 mmol/kg dry wt (dw), P < 0.05 (Table 3)]. With the exception of modest perturbations in BCAA metabolism, there were no additional changes in amino acid metabolism with glutamate infusion (Table 2).

Effects of training and glutamate infusion on TCA cycle intermediates, phosphocreatine, lactate, and glucose metabolism. Under control conditions at peak exercise, the UT thigh demonstrated a significant drop in 2-oxoglutarate (peak exercise vs. rest: 12.2 ± 1.6 vs. 32.4 ± 6.8 μmol/kg dw, P < 0.05; Fig. 4A) and a concomitant increase in both malate and fumarate compared with rest (malate: 2.2 ± 0.4 vs. 0.5 ± 0.3 mmol/kg dw, P < 0.05, Fig. 4B; fumarate: 0.33 ± 0.07 vs. 0.02 ± 0.01 mmol/kg dw, P < 0.05, Fig. 4C). With training, the T thigh did not experience a significant drop in 2-oxoglutarate or a rise in malate and fumarate at peak exercise. Training also resulted in an attenuated increase in muscle lactate compared with the UT thigh (P = 0.06; Fig. 5A), and lactate release was elevated to the same degree in both thighs.

Fig. 2. Muscle glutamate concentration is the same for both thighs at rest. At peak exercise, muscle glutamate drops to the same extent in both thighs under control conditions and with glutamate infusion. Data are means ± SE, dw, dry wt. †Statistical significance (P < 0.05) from control rest.

Fig. 3. The flux of key nitrogen carriers including alanine, glutamine, glutamate, branched-chain amino acids (BCAA), and ammonia were compiled to demonstrate relative uptake/release of these compounds at rest and peak exercise for both thighs under control conditions and with glutamate infusion.
Phosphocreatine was reduced to the same extent in both thighs at peak work rate, negating any relationship between TCA cycle intermediates and peak oxidative capacity (Fig. 5B). Arterial glucose concentrations were lower at peak exercise relative to resting conditions in both thighs, which was accompanied by a relatively small increase in the glucagon-to-insulin ratio at peak exercise for the T thigh (Table 4). However, glucose uptake was elevated to the same extent in both thighs at peak work rate.

With glutamate infusion, the differences observed in muscle metabolites between the UT and T thighs under control conditions were no longer evident. Muscle fumarate, malate, and lactate rose, while 2-oxoglutarate as well as phosphocreatine dropped to the same extent in both thighs (Figs. 4 and 5). Both thighs demonstrated increased lactate release and glucose uptake during peak exercise, but this was not different from control conditions. However, arterial lactate was lower with glutamate infusion compared with control during peak exercise for both thighs (Table 2), suggesting that lactate clearance was enhanced. Arterial glucose concentrations were maintained at rest during glutamate infusion but were significantly decreased, while still within normal range, during peak exercise for both UT and T thighs. Glucagon levels were elevated almost twofold compared with control conditions, resulting in the increase in glucagon-to-insulin ratios during the glutamate infusion (Table 4).

DISCUSSION

Despite the finding that peak oxidative capacity was greater in the T thigh compared with the UT thigh, only the UT thigh demonstrated a significant increase in TCA cycle intermediates at peak work rate, negating any relationship between TCA cycle intermediates and peak oxidative capacity (Fig. 5B). Arterial glucose concentrations were lower at peak exercise relative to resting conditions in both thighs, which was accompanied by a relatively small increase in the glucagon-to-insulin ratio at peak exercise for the T thigh (Table 4). However, glucose uptake was elevated to the same extent in both thighs at peak work rate.

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Glutamate, TCA Cycle, and Oxidative Metabolism

Table 4. Insulin and glucagon concentrations and glucagon-to-insulin molar ratios during control and glutamate trials

<table>
<thead>
<tr>
<th></th>
<th>UT Thighs</th>
<th>T Thighs</th>
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<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Control peak work rate</td>
</tr>
<tr>
<td>Insulin, pM</td>
<td>127.4±14.9</td>
<td>73.5±17.4‡</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>77.3±9.6</td>
<td>73.1±9.2</td>
</tr>
<tr>
<td>Glucagon-to-insulin ratio</td>
<td>0.2±0.02</td>
<td>0.4±0.1</td>
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</table>

Data are means ± SE. *Significant difference (P < 0.05) between UT and T thighs at same time point and same conditions. Statistical differences within same thigh (P < 0.05): †vs. control rest, ‡vs. control peak work rate, §vs. glutamate rest.

Oxidative capacity does not relate to TCA cycle anaplerosis.

Five weeks of endurance training of the quadriceps muscle of one thigh resulted in a greater \( \text{V}^\text{O}_2\text{peak} \) and a larger muscle mass compared with the contralateral, untrained quadriceps muscle. However, contrary to the theory that TCA cycle anaplerosis relates positively with TCA cycle flux (26, 30, 31), greater \( \text{V}^\text{O}_2\text{peak} \) in the T thigh was observed in the absence of a significant rise in the TCA cycle intermediates fumarate and malate. With this discrepancy, the expansion of the TCA cycle would not be a determinant of peak oxidative metabolism.

Since peak concentrations of TCA cycle intermediates are normally achieved within the first 5–15 min of exercise (8), we expect that we attained peak concentrations. Our findings are in accordance with previous studies that have shown an attenuated rise in TCA cycle intermediates following training near the onset of submaximal exercise (7, 19).

Training is thought to improve the balance between pyruvate production and oxidation by reducing glycolysis and ultimately attenuate the rise in the TCA intermediate pool. Although we did not measure flux through PDH, LeBlanc et al. (22) showed a decrease in pyruvate flux through the PDH reaction following training. Consistent with this, the present study pyruvate flux through the lactate dehydrogenase reaction was reduced since there was a 30% lower lactate accumulation in the trained compared with the untrained muscle. We also demonstrated that alanine release was significantly elevated and that 2-oxoglutarate was not significantly reduced in the trained muscle at peak exercise compared with the untrained muscle, suggesting that pyruvate flux was elevated through the AAT reaction. While Howarth et al. (19) suggested that pyruvate flux through AAT is reduced in trained muscle secondary to an attenuated accumulation of muscle alanine, they also demonstrated that maximal activity of AAT was elevated with training. Thus it is possible that trained muscle may preferentially direct pyruvate through the AAT reaction to increase alanine production and, ultimately, alanine release.

With increased glutamate availability, trained muscle demonstrated further increases in fumarate and malate concentrations, while 2-oxoglutarate decreased to the same extent as in the untrained muscle without significantly altering \( \text{V}^\text{O}_2\text{peak} \). This is in accordance with a previous study (6) in which glutamine supplementation increased intramuscular glutamate and further expanded the TCA cycle intermediate pool; however, oxidative energy production was unaltered. While the distribution of enhanced glutamate uptake into the mitochondria was unknown, the present data demonstrate a dissociation between TCA cycle anaplerosis and peak oxidative capacity.

Glutamate is intimately related with TCA cycle intermediates and extramuscular metabolism.

Muscle glutamate appears to have an integral role in the TCA cycle and in amino acid (intermediary) metabolism. In contrast to untrained muscle under control conditions, glutamate uptake as well as alanine and BCAA release were elevated in the T thigh, and this was likely due to increased flux through the AAT and BCAA transferase reactions. Both these reactions generate 2-oxoglutarate, which may explain the lack of significant reduction in 2-oxoglutarate at peak exercise in the T thigh. While Howarth et al. (19) observed attenuated changes in muscle glutamate and alanine concentrations between trained and untrained muscle during exercise, our findings revealed an increased glutamate uptake and alanine release in the T thigh compared with the UT thigh in the absence of intramuscular changes. The differences between these studies may potentially be explained by various aspects including the shorter duration of the training protocol used in the present study. The study by Howarth et al. also differed in that submaximal (80% of pretraining \( \text{V}^\text{O}^{\text{max}} \)) cycling exercise was employed rather than peak exercise as an end point for knee extensor exercise.

The further rise in fumarate and malate in the T thigh during glutamate infusion in the present study occurred in the absence of a corresponding increase in alanine release, implying that excess glutamate was not directed through the AAT reaction in the T thigh. One might suggest that prior exercise may have accelerated the increase in PDH activity and reduced the flux of pyruvate through AAT as shown by Gurd et al. (17); unlike Gurd et al., who provided 6 min of rest between exercise protocols, our subjects rested for >1 h before exercising with glutamate infusion. Moreover, the untrained muscle demonstrated an increase in alanine release in the final exercise bout, which would discount the idea that flux through PDH was accelerated with consecutive exercise bouts. Previous work using oral glutamate supplementation (25) as well as intrave-
nous supplementation (29) has also shown attenuated alanine release, independent of repeated exercise.

Muscle glutamate is central to several other transamination reactions that interlink with the TCA cycle, and increased glutamate uptake in the T thigh may have induced a further rise in fumarate and malate through these other transamination reactions. In contrast, enhanced glutamate uptake in the untrained muscle was not directed toward a further increase in TCA cycle intermediates; rather, glutamate was diverted toward synthesis of alanine, which was released by the untrained muscle. Enhanced glutamate uptake may have also been diverted toward glutamine synthesis without entering the TCA cycle, as there was an increase in muscle glutamine with the infusion. These findings suggest that there may be distinct roles for glutamate in untrained compared with trained muscle, which may relate to the magnitude of anaerobiosis during peak exercise.

Glutamate infusion was a useful tool in advancing our understanding of the role of glutamate in intermediary and energy metabolism in muscle as well as its role in extramuscular metabolism. Arterial glutamate was elevated four- to fivefold at rest but was at about half of this concentration during peak exercise in the T thigh. Glutamate infusion enhanced glutamate uptake at rest in both thighs, but uptake was not elevated in the T thigh during peak exercise. This suggests that extramuscular tissues were likely clearing excess circulating glutamate during peak exercise. During the infusion at peak exercise, an approximately twofold increase in glucagon concentration, elevated glucagon-to-insulin ratios, and a drop in arterial lactate (implying enhanced lactate clearance) would be indicative of a rise in gluconeogenesis. However, the lack of rise in circulating glucose or muscle glucose uptake may suggest that glucagon may have stimulated other processes such as lipolysis. These data warrant further investigation to better understand the effects of glutamate on substrate metabolism.

Methodological limitations. While the findings and study design of this work are highly innovative, there are a few limitations that require careful consideration. One might suggest that an order effect exists because control conditions were performed before glutamate infusion. Although the half-life for glutamate is ~10 min (21), we wanted to avoid the risk of contamination of our control conditions. We provided ample rest between exercise periods so that previous exercise would not confound our results. This is confirmed by the consistent arterial glutamate concentrations at rest before exercise for either UT or T thighs. Despite the fact that exercise duration was short for the control condition, muscle biopsies before the glutamate infusion would have confirmed whether muscle glutamate concentrations were reestablished during the rest period. Performing these experiments on different days would have affected training status of the T thigh, and it would not be considered ethical to reininsert femoral catheters on a consecutive day. With a primary focus on peak exercise and the metabolic differences revealed between the UT and T thighs, resting blood flow measures were performed on one subject. While this may be considered a limitation, resting blood flow values are consistent, with standard errors of ~0.01 L·min⁻¹·kg⁻¹ (5, 10, 11).

There may also be some concern with the relatively small sample size used in the present study. Although seven subjects completed the training protocol, complete data in the T and UT thighs with and without glutamate infusion were only obtained on five subjects because of the invasiveness and technical challenges of this study. Despite this sample size, the unique design of this study and the precise techniques for quantification of the metabolism of human muscle provided clear and internally consistent results, demonstrating the usefulness of glutamate supplementation as an elemental tool for future work.

Conclusions. While it has been purported that the anaerobiosis of the TCA cycle is necessary to drive oxidative potential (26, 30, 31), we demonstrated that oxidative capacity at peak exercise in trained muscle is dissociated from TCA cycle anaerobiosis. With enhanced glutamate availability, fumarate and malate were further elevated in the T thigh compared with peak exercise under control conditions, but this did not alter peak oxidative capacity. With the use of glutamate infusion as a tool to examine the dynamic changes in amino acids during peak exercise, distinct relationships between amino acids and the TCA cycle were revealed in trained and untrained muscle.

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