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Enhanced muscle glucose uptake facilitates nitrogen efflux from exercised muscle

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Galassetti, Pietro, Fiona K. Gibbons, Katherine S. Hamilton, D. Brooks Lacy, Alan D. Cherrington, and David H. Wasserman. Enhanced muscle glucose uptake facilitates nitrogen efflux from exercised muscle. *J. Appl. Physiol.* 84(6): 1952–1959, 1998.—The hypothesis that glucose ingestion in the postexercise state enhances the synthesis of glutamine and alanine in the skeletal muscle was tested. Glucose was infused intraduodenally for 150 min ($44.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) beginning 30 min after a 150-min period of exercise ($n = 7$) or an equivalent duration sedentary period ($n = 10$) in 18-h-fasted dogs. Prior exercise caused a twofold greater increase in limb glucose uptake during the intraduodenal glucose infusion compared with uptake in sedentary dogs. Arterial glutamine levels fell gradually with the glucose load in both groups. Net hindlimb glutamine efflux increased in response to intraduodenal glucose in exercised but not sedentary dogs ($P < 0.05$ – 0.01). Arterial alanine levels, depleted by 50% with exercise, rose with intraduodenal glucose in exercised but not sedentary dogs ($P < 0.05$ – 0.01). Net hindlimb alanine efflux also rose in exercised dogs in response to intraduodenal glucose ($P < 0.05$ – 0.01), whereas it was not different from baseline in sedentary controls for the first 90 min of glucose infusion. Beyond this point, it, too, rose significantly. We conclude that oral glucose may facilitate recovery of muscle from prolonged exercise by enhancing the removal of nitrogen in the form of glutamine and alanine.

glutamine; alanine; dog; exercise recovery; hindlimb

AMMONIA is formed at an increased rate in working muscle caused by the deamination of branched-chain amino acids (BCAA) and adenine nucleotides (5, 17). Exceedingly high ammonia concentrations may interfere with proper muscle function. Several mechanisms exist for removal of ammonia from the working muscle. Some ammonia is released from the working muscle as free ammonia (6, 12). More ammonia is released as alanine (4, 6, 12, 13) and glutamine (2, 6, 12, 13) after reaction with pyruvate and glutamate, respectively. Although most of the ammonia is effectively removed from the working muscle, some still accumulates (6, 12, 13). This suggests that its release as a free molecule may be limited. On the other hand, its release as a part of glutamine and, possibly to a smaller extent, of alanine may, in turn, be limited by the muscle cells' ability to form a sufficient amount of these amino acids, at least under moderate-exercise conditions. Support for this view comes from the fact that muscle ammonia accumulates during exercise but that alanine and glutamine generally do not (6, 12, 13).

It is well known that muscle glucose uptake by the working muscle during a glucose load is increased in the postexercise state (18). The main fate of this added

glucose uptake after exercise is deposition as muscle glycogen (18). The hypothesis tested by the present studies is that an additional function of the elevated muscle glucose uptake after exercise is to provide carbons that can facilitate the formation of glutamine, and possibly alanine, and the removal of nitrogen. This hypothesis was tested by measuring the net release of glutamine and alanine in a chronically catheterized dog model that was studied during an intraduodenal glucose load after prolonged exercise or during an equivalent sedentary period.

METHODS

Animal maintenance and surgical procedures. Mongrel dogs of either gender were studied ($n = 17$; mean wt, 20.4 ± 0.5 kg). They had been fed a standard diet [KalKan beef dinner (Vernon, CA); and Wayne Lab Blox, (Allied Mills, Chicago, IL): 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry weight]. The dogs were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the protocols were approved by Vanderbilt University's Institutional Animal Care and Use Subcommittee. At least 16 days before each experiment, a laparotomy was performed while the dogs were under general anesthesia (0.04 mg/kg of atropine and 15 mg/kg pentobarbital sodium before surgery, and 1.0% isoflurane inhalation anesthetic during surgery). A Silastic catheter (0.08-in. ID) was inserted through the duodenal mucosa 3–4 cm below the pylorus for infusion of glucose and was secured with a purse-string suture. Silastic catheters (0.04-in. ID) were inserted into the portal vein and left common hepatic vein for blood sampling. Incisions were also made in the neck region and inguinal region for the placement of arterial and common iliac vein sampling catheters, respectively. The carotid artery was isolated, and a Silastic catheter (0.04-in. ID) was inserted so that its tip rested in the aortic arch. A Silastic catheter (0.03-in. ID) was introduced into the common iliac vein via a lateral circumflex vein. Exposure of the lateral circumflex vein was achieved with a 2-cm incision in the lower femoral region, and the vein was dissected from the subcutaneous tissues. The catheter tip was positioned in the common iliac vein, distal to the anastomosis with the vena cava. The median sacral vein was ligated to prevent dilution from other sites. Verification of catheter placement was made through the abdominal incision site. After the catheters were inserted, they were filled with saline containing heparin (200 U/ml; Abbott Laboratories, North Chicago, IL), and their free ends were knotted.

Doppler flow probes (Instrumentation Development Laboratory, Baylor University School of Medicine, Houston, TX; or Transonic Systems, Ithaca, NY) were used to measure blood flows in the portal vein (PVF), hepatic artery (HAF), and external iliac artery (9). Briefly, a small section of the portal vein, upstream from its junction with the gastroduodenal vein, was cleared of tissue, and a cuff was placed around the vessel and secured. The gastroduodenal vein was isolated and

then ligated proximal to its coalescence with the portal vein. A section of the main hepatic artery lying proximal to the portal vein was isolated, and a flow cuff was placed around the vessel and secured. The external iliac artery was accessed from the abdominal incision, dissected free of surrounding tissue, and fitted with a flow probe cuff that was then secured around the vessel. The Doppler probe leads and the knotted free catheter ends, with the exception of the carotid artery and common iliac vein catheters, were stored in a subcutaneous pocket in the abdominal region so that complete closure of the skin incision was possible. The carotid artery and the common iliac vein catheters were stored under the skin of the neck and inguinal regions, respectively.

Starting 1 wk after surgery, regardless of whether they were used for sedentary or exercise experiments, dogs were exercised on a motorized treadmill so that they would be familiar with treadmill running. Animals were not exercised during the 48 h preceding an experiment. Only animals that had a leukocyte count $<18,000/\text{mm}^3$, normal stools, and a good appetite (consuming all of the daily ration) were used.

On the day of the experiment, the subcutaneous ends of the catheters were freed through small skin incisions made in the abdominal and neck regions under local anesthesia (2% lidocaine, Astra Pharmaceutical Products, Worcester, MA). The contents of each catheter were aspirated, and the catheters were flushed with saline. Silastic tubing was connected to the exposed catheters and brought to the back of the dog, where the catheters were secured with quick-drying glue. Saline (0.1 ml/min) was infused in the arterial catheter throughout experiments.

Experimental procedures. Dogs, fasted for 18 h, were either exercised at a moderate intensity (100 m/min, 12% grade) on a motorized treadmill ($n = 7$ for arterial and limb balance measurements; catheter failure resulted in $n = 6$ for liver balance measurements) or remained sedentary ($n = 10$ for limb balances, $n = 8$ for liver balances) for a corresponding time period ($t = -180$ to -30 min). The exercise intensity used in these experiments has been shown to result in a twofold increase in heart rate (20), an increase in O_2 uptake to $\sim 50\%$ of maximum (15), and a fall of $\sim 70\%$ in liver glycogen (21). A period of exercise recovery or continued rest followed ($t = -30$ to 150 min). The first 20 min of the postexercise period were required to reestablish a baseline for alanine and glutamine measurements. Baseline measurements were made at -10 and 0 min. Glucose (50% dextrose) was given as a primed infusion ($834.0 \mu\text{mol}/\text{kg}$; $44.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) into the duodenum from $t = 0$ to 150 min. Samples were taken at $t = 15, 30, 60, 90, 120,$ and 150 min during the intraduodenal glucose infusion.

Blood flows were recorded continuously from the frequency shifts of the pulsed sound signal emitted from the Doppler flow probes (9). At $t = -70$ min, indocyanine green (ICG; $0.1 \text{ mg} \cdot \text{m}^2 \cdot \text{min}^{-1}$) infusion was initiated to provide a backup measurement of hepatic blood flow and to serve as a means of verification of hepatic vein catheter replacement. Data not pertaining to glutamine and alanine are a subset of data published previously (8) and are so designated in appropriate table legends.

Processing of blood and tissue samples. After they were centrifuged, plasma and deproteinized blood were stored on dry ice until the completion of the experiment. Samples were then stored at -70°C until later analysis. Plasma glucose levels were determined by the glucose oxidase method by using a Beckman glucose analyzer (Beckman Instruments, Fullerton, CA). Whole blood lactate, alanine, and glucose concentrations were determined in samples deproteinized with an equal volume of 8% perchloric acid by enzymatic

methods (11) on a Technicon AutoAnalyzer (Tarrytown, NY) or on a Monarch 2000 centrifugal analyzer (Instrumentation Laboratories, Lexington, MA). For the measurement of glutamine, an aliquot of deproteinized blood from each sample was placed in each of two tubes containing 0.2 M sodium acetate buffer. In one tube, glutamine was completely reacted to glutamate with glutaminase. The amount of glutamate was then determined in both samples by measuring fluorometrically, on a Technicon AutoAnalyzer, the conversion of NAD to NADH after the reaction catalyzed by glutamate dehydrogenase. The glutamine concentration was determined as the difference in glutamate concentrations between the tube that was reacted with glutaminase and the tube that was not. Enzymes used in analyses of metabolites and amino acids were obtained from Sigma Chemical (St. Louis, MO) or Boehringer-Mannheim Biochemicals (Mannheim, Germany).

Immunoreactive insulin was measured by using a double-antibody system (interassay coefficient of variation, 10%) (14). Immunoreactive glucagon (3,500 mol wt) was measured in plasma samples containing 50 μl of 500 kallikrein-inactivating units/ml Trasylol (FBA Pharmaceuticals, New York, NY) by using a double-antibody system (interassay coefficient of variation, 7%) modified from the method developed by Morgan and Lazarow for insulin (14). Glucagon and insulin antisera were obtained from Dr. R. L. Gingerich, Washington University School of Medicine (St. Louis, MO), and the standard glucagon and ^{125}I -labeled glucagon were obtained from Novo Research Institute (Copenhagen, Denmark). Standard insulin and ^{125}I -labeled insulin were obtained from Linco Research (St. Louis, MO).

Calculations. Net limb balances were calculated as $\text{LF} \times ([\text{I}] - [\text{A}])$, where LF is limb blood flow through the external iliac artery, and [I] and [A] are the substrate levels in the common iliac vein and arterial blood, respectively. The sign was reversed for the calculation of net limb glucose balance so that net uptake would be positive. Although intended to represent mostly substrate balance in skeletal muscle, hindlimb balances actually indicate total hindlimb balances. In experimental animals of the size used in our study, one hindlimb weighs on average $\sim 1,000$ g, of which skeletal muscle is ~ 600 g, or $\sim 60\%$. Thus, an estimate of a substrate balance per kilogram of muscle tissue can be made by dividing the given hindlimb balance value by 0.6 (19).

Blood levels and flows were used for the calculation of all hepatic and limb balances. The ratio of blood to plasma glucose was calculated for the basal period and for the glucose infusion period for each dog at each sampling site. Plasma glucose values were then multiplied by their corresponding ratio (i.e., blood glucose/plasma glucose) to convert to blood glucose concentrations. The advantage of using plasma glucose measurements is that a large number of replicates can be done quickly and at little added cost. The conversion to blood values alleviates the need for assumptions regarding the equilibration of substrates between red cell and plasma water.

Net hepatic balances were determined by the formula $\text{HAF} \times ([\text{A}] - [\text{H}]) + \text{PVF} \times ([\text{P}] - [\text{H}])$, where [P] and [H] are the portal vein and hepatic vein substrate concentrations, respectively. Net gut glutamine balance was calculated as $\text{PVF} \times ([\text{A}] - [\text{P}])$. The sign was reversed for calculation of net gut alanine balance. Doppler-determined blood flow measurements were used, with the exception of one experiment, when probe failure required the use of results obtained by using the dye-extraction method. The dye-extraction technique measures total hepatic blood flow but does not differentiate between inputs from the portal vein and hepatic artery. In the

experiment that was reliant on the dye-extraction technique, PVF was assumed to be 80% of total hepatic blood flow (7).

Statistical analysis was performed by using SuperAnova (Abacus Concepts, Berkeley, CA) on a Macintosh PowerPC. Statistical comparisons between groups and over time were made by using ANOVA designed to account for repeated measures. Specific time points were examined for significance by using contrasts solved by univariate repeated measures. Statistics are reported in the corresponding table or figure legend for each variable. Differences were considered significant when P values were <0.05 . Data are expressed as means \pm SE.

RESULTS

Arterial plasma insulin and glucagon. Baseline arterial plasma insulin was 7 ± 1 $\mu\text{U/ml}$ in exercised dogs and 8 ± 2 $\mu\text{U/ml}$ in sedentary dogs. All dogs responded similarly to intraduodenal glucose, with values of 27 ± 4 and 25 ± 4 $\mu\text{U/ml}$ in exercised and sedentary animals, respectively, at 150 min. Arterial glucagon was higher in the baseline period before intraduodenal glucose in the exercised dogs (63 ± 6 pg/ml) compared with baseline concentrations in the sedentary dogs (40 ± 1 pg/ml, $P < 0.01$). Arterial glucagon levels in exercised and sedentary dogs converged during the intraduodenal glucose infusion as levels fell gradually in exercised dogs ($P < 0.05$ – 0.01 vs. baseline between 60 and 150 min). Values at 150 min were 47 ± 6 pg/ml in exercised and 38 ± 5 pg/ml in sedentary animals.

Arterial plasma glucose levels and net limb glucose uptake. Baseline arterial glucose concentrations were not significantly different in exercised and sedentary dogs (5.8 ± 0.2 and 6.0 ± 0.1 mM, respectively). Arterial plasma glucose rose more in exercised dogs than in sedentary dogs in response to intraduodenal glucose. This led to consistently higher arterial plasma glucose values during glucose infusion (at 150 min, values were 7.8 ± 0.2 and 8.9 ± 0.6 mM in sedentary and exercised dogs, respectively; $P < 0.05$). Rates of net limb glucose uptake were already higher in exercised dogs (56 ± 17 $\mu\text{mol/min}$) than in sedentary (30 ± 9 $\mu\text{mol/min}$, $P < 0.05$) dogs in the baseline period. The increase in net limb glucose uptake was approximately twofold higher in exercised dogs than the increase present in sedentary dogs during the intraduodenal glucose infusion period (at 150 min, values were 205 ± 39 and 105 ± 14 $\mu\text{mol/min}$, respectively).

Arterial blood glutamine levels, net limb glutamine output, net hepatic glutamine balance, and net gut glutamine uptake. Arterial blood glutamine levels were similar in exercised and sedentary dogs (765 ± 62 vs. 722 ± 69 μM , respectively) in the baseline period before intraduodenal glucose. Arterial blood glutamine fell significantly in both exercised dogs (decrease of 104 ± 33 μM at $t = 150$ min) and sedentary dogs (decrease of 186 ± 30 μM at $t = 150$ min) in response to the intraduodenal glucose load (Fig. 1). The decrement in arterial glutamine levels during intraduodenal glucose in sedentary dogs was greater than the decrement after exercise ($P < 0.02$ – 0.01 at $t = 120$ and 150 min). There was no significant difference in baseline rates of net limb glutamine output in exercised and sedentary dogs

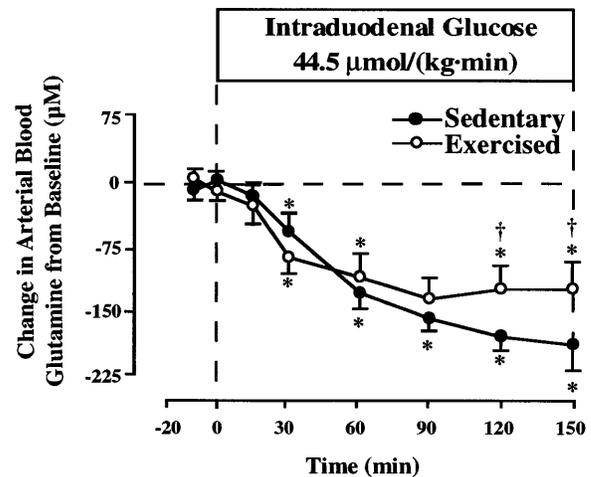


Fig. 1. Change in arterial blood glutamine concentration in response to intraduodenal glucose infusion ($44.5 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) in sedentary ($n = 6$) and exercised ($n = 7$) dogs. Data are means \pm SE. *Significant change from baseline concentrations, $P < 0.05$. †Significant difference from change at corresponding sedentary time point, $P < 0.05$.

(4.4 ± 3.8 vs. 5.6 ± 2.2 $\mu\text{mol/min}$, respectively). In response to the intraduodenal glucose load, net limb glutamine output increased rapidly and remained elevated in exercised dogs (18.5 ± 4.9 , 17.7 ± 6.6 , and 14.3 ± 4.9 $\mu\text{mol/min}$ at 30, 90, and 150 min, respectively; Fig. 2). Conversely, net limb glutamine output remained essentially unchanged in sedentary dogs for the first 90 min of glucose infusion (6.0 ± 2.7 and 5.2 ± 2.6 $\mu\text{mol/min}$ at 30 and 90 min, respectively; Fig. 2), whereas it increased at the end of the 150-min glucose infusion period (13.3 ± 5.5 $\mu\text{mol/min}$ at 150 min; Fig. 2). Net limb glutamine output rose significantly more in exercised compared with sedentary dogs at $t = 30$ and 90 min ($P < 0.05$).

Sedentary dogs tended to exhibit net hepatic output of glutamine during the baseline and intraduodenal glucose infusion periods, whereas exercised dogs tended

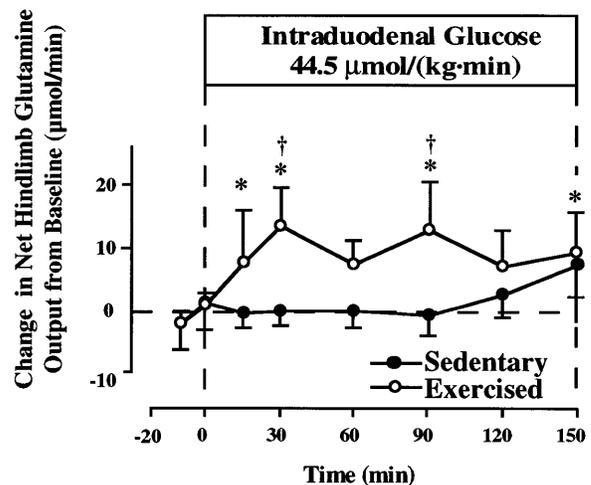


Fig. 2. Change in net hindlimb glutamine output in response to intraduodenal glucose infusion ($44.5 \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$) in sedentary ($n = 6$) and exercised ($n = 7$) dogs. Data are means \pm SE. *Significant change from baseline rates, $P < 0.05$. †Significant difference from change at corresponding sedentary time point, $P < 0.05$.

Table 1. Net hepatic balance and gut uptake of glutamine

Condition	Baseline	Intraduodenal Glucose ($44.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)					
		15 min	30 min	60 min	90 min	120 min	150 min
<i>Net hepatic glutamine balance, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$</i>							
Sedentary	-0.6 ± 0.1	-0.9 ± 0.6	-0.1 ± 0.5	0.1 ± 0.5	-0.7 ± 0.5	-1.2 ± 0.5	-0.9 ± 0.6
Exercised	0.3 ± 0.3	1.2 ± 0.6	0.7 ± 0.3	0.3 ± 0.4	0.4 ± 0.4	0.1 ± 0.3	0.1 ± 0.3
<i>Net gut glutamine uptake, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$</i>							
Sedentary	1.4 ± 0.2	2.2 ± 0.7	1.0 ± 0.5	1.1 ± 0.1	1.2 ± 0.3	1.4 ± 0.7	0.9 ± 0.2
Exercised	1.7 ± 0.3	1.6 ± 0.4	1.5 ± 0.2	2.4 ± 0.3	1.4 ± 0.3	2.1 ± 0.2	1.7 ± 0.5

Values are means \pm SE; $n = 8$ and 6 , sedentary and exercised dogs, respectively. Negative net hepatic balance values indicate net output.

to display net hepatic glutamine uptake (Table 1). Rates were significantly different between the two groups during the baseline period and at 15 min of intraduodenal glucose infusion ($P < 0.05$). Net gut glutamine uptake was not significantly affected by intraduodenal glucose in either sedentary or exercised dogs. In addition, rates of net gut glutamine uptake were not different between groups (Table 1).

Arterial blood alanine levels, net limb alanine output, net hepatic alanine uptake, and net gut alanine uptake. Arterial blood alanine levels in the baseline period were reduced in exercised ($186 \pm 26 \mu\text{M}$) compared with sedentary dogs ($318 \pm 24 \mu\text{M}$). In response to intraduodenal glucose, arterial alanine levels rose by $96 \pm 22 \mu\text{M}$ at $t = 150$ min in exercised dogs ($P < 0.05$ – 0.01 at $t = 90$ to 150 min; Fig. 3). In contrast, arterial alanine levels remained unchanged in response to intraduodenal glucose ($-17 \pm 29 \mu\text{M}$ at $t = 150$ min in sedentary dogs; $P < 0.05$ – 0.02 vs. exercised dogs throughout the whole glucose infusion period for changes over baseline; Fig. 3). Baseline net limb alanine output was similar in exercised ($1.3 \pm 0.8 \mu\text{mol}/\text{min}$) and sedentary ($1.5 \pm 1.3 \mu\text{mol}/\text{min}$) dogs. Net limb alanine output was increased significantly after 30 min of intraduodenal glucose in exercised dogs and remained elevated until the end of the experiment, averaging 4.6 ± 1.2 , 5.1 ± 1.5 , and $6.4 \pm 1.6 \mu\text{mol}/\text{min}$ at the 30-, 90-, and 150-min

time points ($P < 0.05$ – 0.01 ; Fig. 4). In contrast, net limb alanine output was not affected by intraduodenal glucose in sedentary dogs for the first 90 min of glucose infusion (1.3 ± 1.1 vs. $1.4 \pm 2.0 \mu\text{mol}/\text{min}$ at 60 and 90 min, respectively; Fig. 4). Subsequently, it rose significantly (to $5.4 \pm 2.1 \mu\text{mol}/\text{min}$ at 120 min and to $7.2 \pm 1.3 \mu\text{mol}/\text{min}$ at 150 min; $P < 0.05$).

Net hepatic alanine uptake was not significantly affected by intraduodenal glucose in either exercised or sedentary dogs (Table 2). In addition, rates between groups were not different during either the baseline or intraduodenal glucose infusion periods. Net gut alanine output was unaffected by intraduodenal glucose in sedentary and exercised dogs. There was no significant difference between groups during the baseline or intraduodenal glucose infusion periods (Table 2).

Blood glutamate levels and net limb glutamate balance. Arterial and iliac venous blood glutamate concentrations (Table 3) were similar during the baseline and glucose infusion periods in exercised and sedentary dogs. Baseline net limb glutamate uptake was also similar in exercised and sedentary dogs. In both groups, net limb glutamate uptake increased by $\sim 40\%$ during glucose infusion (Table 3).

Arterial blood lactate levels and net limb lactate balance. Arterial blood lactate was similar during the baseline period in exercised and sedentary dogs ($473 \pm$

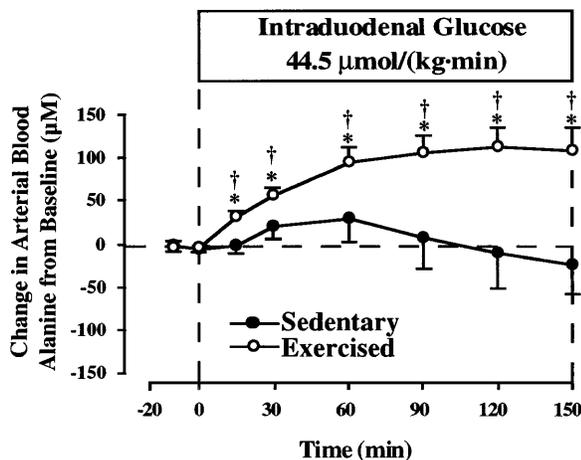


Fig. 3. Change in arterial blood alanine concentration in response to intraduodenal glucose infusion ($44.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in sedentary ($n = 6$) and exercised ($n = 7$) dogs. Data are means \pm SE. * Significant change from baseline concentrations, $P < 0.05$. † Significant difference from change at corresponding sedentary time point, $P < 0.05$.

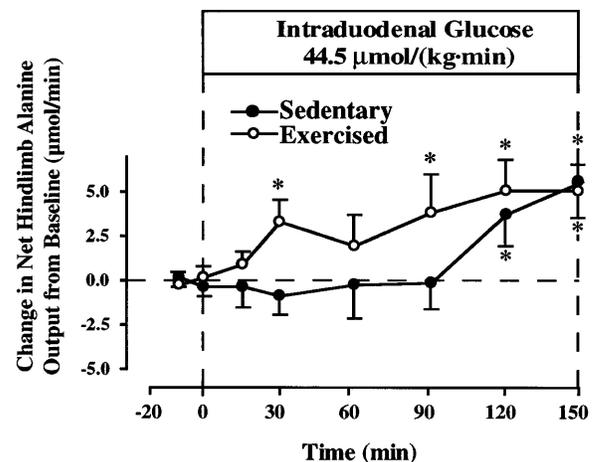


Fig. 4. Change in net hindlimb alanine output in response to intraduodenal glucose infusion ($44.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) in sedentary ($n = 6$) and exercised ($n = 7$) dogs. Data are means \pm SE. * Significant change from baseline rates, $P < 0.05$.

Table 2. Net hepatic uptake and gut output of alanine

Condition	Baseline	Intraduodenal Glucose (44.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)					
		15 min	30 min	60 min	90 min	120 min	150 min
<i>Net hepatic alanine uptake, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$</i>							
Sedentary	2.2 \pm 0.3	1.7 \pm 0.7	2.4 \pm 0.5	2.0 \pm 0.3	2.0 \pm 0.3	2.2 \pm 0.3	2.1 \pm 0.2
Exercised	2.4 \pm 0.3	2.7 \pm 0.4	2.5 \pm 0.3	2.9 \pm 0.3	2.7 \pm 0.2	3.1 \pm 0.3	2.5 \pm 0.2
<i>Net gut alanine output, $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$</i>							
Sedentary	0.9 \pm 0.1	1.1 \pm 0.3	1.1 \pm 0.4	0.8 \pm 0.4	1.0 \pm 0.3	1.1 \pm 0.3	0.8 \pm 0.5
Exercised	0.9 \pm 0.2	1.6 \pm 0.4	1.4 \pm 0.3	1.1 \pm 0.3	1.0 \pm 0.2	1.0 \pm 0.3	0.8 \pm 0.1

Values are means \pm SE; $n = 8$ and 6 in sedentary and exercised dogs, respectively.

49 and $567 \pm 53 \mu\text{M}$, respectively). Both groups were also characterized by similar increments in arterial lactate in response to the glucose load (at 150 min, 858 ± 90 and $858 \pm 80 \mu\text{M}$ in exercised and sedentary dogs, respectively). Baseline net limb lactate balance was essentially zero in both groups. The limb became a marked net consumer of lactate in response to an intraduodenal glucose load in both exercised and sedentary animals. Rates of net limb lactate uptake were similar during the glucose load in both groups (at 150 min, 30 ± 7 and $20 \pm 6 \mu\text{mol}/\text{min}$ in exercised animals in sedentary animals, respectively).

Hindlimb blood flow. External iliac artery blood flow (Table 4), a marker of hindlimb blood flow, was not affected by the intraduodenal glucose load in either group and was not significantly different between groups. Similarly, PVF and HAF (Table 4) were unchanged by intraduodenal glucose. Moreover, there were no differences between groups.

DISCUSSION

It is well known that skeletal muscle glucose uptake in response to oral glucose is increased after exercise. These results suggest that the added glucose consumed by muscle after exercise provides carbon substrate that facilitates the removal of previously accumulated nitrogen groups. The result is an increase in the efflux from the skeletal muscle of glutamine and, to a smaller extent, of alanine, in the postexercise state. Limb glucose uptake was increased by $\sim 75 \mu\text{mol}/\text{min}$ in the sedentary dogs and by $\sim 150 \mu\text{mol}/\text{min}$ in the exercised

dogs during the intraduodenal glucose infusion period. Limb glutamine output could account for $\sim 13\%$ of the extra glucose consumed in working muscle. Limb alanine efflux was also increased after exercise and could have accounted for $\sim 5\%$ of the limb glucose uptake. It has been shown that limb glucose uptake is increased in response to an oral glucose load caused by increased nonoxidative glucose metabolism. The increased nonoxidative glucose metabolism is largely reflected in an increase in muscle glycogen deposition. A fraction of the added glucose taken up by muscle also appears to be metabolized and transaminated, forming amino acids that are then readily released from the muscle.

The increase in glutamine and alanine efflux from the skeletal muscle after exercise raises the issue of the source of the amino groups they transport. A circulatory origin of the amino groups while separate muscle groups are exercised could be suggested by the findings of Bangsbo et al. (1), who observed net ammonia uptake in the resting skeletal muscles. Eriksson et al. (2) have reported that, in humans, leg muscles display net ammonia uptake at rest, shift to net production during exercise, and shift back to net uptake only 30–60 min after the end of exercise. Because we did not measure ammonia balances in the present study, we do not know whether, in this experimental setting, ammonia was taken up or produced by the hindlimb muscle. Nevertheless, if net uptake was present, the excess ammonia taken up could likely represent only a fraction of the amino groups excreted as glutamine and alanine. For instance, a fractional ammonia extraction of 50%, with

Table 3. Glutamate arterial and common iliac venous concentrations and net glutamate hindlimb balance

Condition	Baseline	Intraduodenal Glucose (44.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)					
		15	30	60	90	120	150
<i>Arterial glutamate, μM</i>							
Sedentary	69 \pm 8	70 \pm 8	70 \pm 6	63 \pm 6	72 \pm 8	70 \pm 7	69 \pm 5
Exercised	88 \pm 13	86 \pm 13	76 \pm 13	72 \pm 12	78 \pm 13	75 \pm 11	77 \pm 13
<i>Common iliac venous glutamate, μM</i>							
Sedentary	62 \pm 7	64 \pm 8	62 \pm 7	58 \pm 7	63 \pm 8	63 \pm 6	60 \pm 5
Exercised	83 \pm 14	75 \pm 14	70 \pm 11	69 \pm 12	71 \pm 13	67 \pm 10	71 \pm 14
<i>Net hindlimb glutamate uptake, $\mu\text{mol}/\text{min}$</i>							
Sedentary	1.21 \pm 0.72	1.50 \pm 0.60	1.86 \pm 0.39	0.82 \pm 0.67	2.00 \pm 0.33	1.62 \pm 0.51	2.19 \pm 0.40
Exercised	1.11 \pm 0.53	1.90 \pm 0.39	1.93 \pm 1.18	0.95 \pm 0.78	1.14 \pm 1.11	2.37 \pm 1.14	1.49 \pm 0.97

Values are means \pm SE; $n = 10$ and 7 in sedentary and exercised dogs, respectively.

Table 4. *Blood flow measurements*

Condition	Baseline	Intraduodenal Glucose (44.5 $\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)					
		15 min	30 min	60 min	90 min	120 min	150 min
<i>External iliac artery, ml/min</i>							
Sedentary	221 \pm 17	223 \pm 16	219 \pm 19	211 \pm 15	204 \pm 16	206 \pm 16	224 \pm 14
Exercised	249 \pm 42	249 \pm 45	253 \pm 45	241 \pm 42	244 \pm 44	247 \pm 40	246 \pm 35
<i>Hepatic artery, ml/min</i>							
Sedentary	99 \pm 18	88 \pm 16	92 \pm 17	90 \pm 17	91 \pm 16	90 \pm 16	85 \pm 16
Exercised	98 \pm 9	89 \pm 10	89 \pm 13	98 \pm 11	97 \pm 12	94 \pm 11	88 \pm 12
<i>Portal vein, ml/min</i>							
Sedentary	474 \pm 75	414 \pm 74	430 \pm 77	418 \pm 75	415 \pm 75	407 \pm 79	379 \pm 68
Exercised	481 \pm 24	454 \pm 55	442 \pm 52	466 \pm 34	454 \pm 34	465 \pm 32	418 \pm 45

Values are means \pm SE; $n = 10$ and 7 in sedentary and exercised dogs, respectively, for external iliac artery flows; 8 and 7 in sedentary and exercised dogs, respectively, for hepatic artery and portal vein blood flows. These values comprise part of database used in Ref. 8.

arterial levels at the upper end of the normal range, could only account for $\sim 3\text{--}4$ $\mu\text{mol}/\text{min}$ of nitrogen groups, whereas the peak increase in glutamine output alone was ~ 13 $\mu\text{mol}/\text{min}$. Therefore, we believe that ammonia of circulatory origin could only supplement a larger "pool" of intracellular amino groups. The latter may be formed in part by ammonia accumulated during exercise. Additional amino groups may be produced after exercise from the same processes that generate ammonia during exercise (accelerated deamination of BCAA and, to some extent, adenine nucleotides), which at the cessation of exercise may still remain above baseline values.

One molecule of glutamate is needed for each glutamine that is produced; the increase in glutamine efflux after exercise therefore implies an increased availability of glutamate. Table 3 shows that the uptake of circulatory glutamate is not increased when glucose is infused after exercise and is not a likely source of the excess glutamate that is required. Sources of glutamate are intramuscular free glutamate, transamination of BCAA, intramuscular protein breakdown, and conversion of α -ketoglutarate derived from glucose oxidation. The latter mechanism, we believe, is the most likely to be influenced by glucose infusion in the postexercise state. The α -ketoglutarate pool is small compared with the amount of glutamate necessary to account for glutamine output. Its turnover may be accelerated by the increased glucose load. The α -ketoglutarate pool may also be increased by the degradation to α -ketoglutarate of arginine and histidine, released during protein breakdown. Unfortunately, our experimental design did not allow to discriminate among the several potential sources of glutamate. Therefore, any conclusion will remain speculative until supported by further work.

The limb switched from being a net lactate producer before the intraduodenal glucose load to a net lactate consumer during the glucose load. This is consistent with studies in human subjects that show that glucose ingestion increases net lactate uptake by the human forearm (10, 16). It is surprising, in a sense, that while prior exercise accentuates the increase in muscle glucose uptake during an intraduodenal glucose load and increases the concurrent efflux of amino acids, it has no

effect on the increase in net lactate uptake. It is possible that the carbons for alanine and, less likely for glutamine formation, in part, originate from lactate. Although prior exercise does not alter the rate of net lactate uptake, prior exercise could conceivably alter the fate of the lactate once inside the muscle cell. From a quantitative standpoint, lactate could conceivably provide the carbons for the released amino acids if one assumes that during the intraduodenal glucose infusion all the lactate consumed by the hindlimb is diverted to alanine (with the intermediate formation of pyruvate) and, less likely, to glutamine, via previous conversion to 2-oxyglutarate and glutamate.

The ability of prior exercise to amplify the release of glutamine during a glucose load may possibly be related to an increase in the activity of glutamine synthase. Results of this study and a previous study (20) show that hindlimb glutamine output tends to be higher after exercise even in the absence of a glucose load. Under those conditions, however, the effect is considerably less than in the presence of a glucose load and varies little during the first 90 min after exercise. It may be that the increase in glucose flux caused by the glucose load is necessary to provide adequate substrate for differences in the flux through the glutamine synthase reaction to be readily detectable. In the dog, glucocorticoid levels increase threefold in response to this exercise protocol (21). Moreover, in the rat, glucocorticoids stimulate glutamine synthase mRNA and protein levels (3). The effect of the glucocorticoids is thought to be delayed, requiring an extended time interval, and the effect may not be evident in the immediate postexercise period after 2.5 h of exercise. In light of the results of the present study, it is interesting that 12–16 wk of exercise training actually leads to a marked reduction in glutamine synthase activity and mRNA in fast-twitch red muscles fibers of the rat (3). It is possible that the stress of acute exercise increases the muscle enzyme activity, whereas the adaptations to chronic exercise result in a suppression.

The fates of the excess glutamine and alanine released by the hindlimb during the intraduodenal glucose load after exercise are not entirely clear. The added alanine produced with intraduodenal glucose in the

postexercise state replenishes the circulating plasma alanine level stores, which were reduced by ~50% at the end of 150 min of exercise. One can estimate that circulating alanine stores were replenished at a rate of $\sim 0.1 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. If one assumes that the muscles of the hindlimb are representative of all skeletal muscle and that one-half the body weight of the dog is skeletal muscle, then it can be calculated that total muscle alanine efflux is $\sim 0.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$. It can, therefore, be roughly estimated that ~50% of the excess alanine produced by skeletal muscle is used to replenish plasma alanine stores. It is possible that alanine uptake by various tissues is increased after exercise. Net hepatic alanine uptake, however, is not significantly greater in the postexercise state compared with the basal state during intraduodenal glucose load (which also suggests that hepatic alanine uptake can be independent of broad variations in alanine circulating levels). It may be that alanine removal is distributed among many tissues, such as kidney or nonworking muscles. The estimated glutamine efflux from total body skeletal muscle is $\sim 1.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ greater in exercised than sedentary dogs in the postexercise state during the glucose load. The circulating glutamine pool size falls slightly less in exercised compared with sedentary dogs. Therefore, a part of the added glutamine released by the hindlimb remains in the blood glutamine pool. Although net gut and hepatic glutamine uptakes tend to be higher after exercise, differences are not significant. Again, it is possible that glutamine removal is distributed so that many tissues are involved and significant uptake is difficult to detect in any one tissue. Of particular importance in the removal of glutamine may be the kidney, where glutamine is deaminated and the nitrogen is excreted.

An alternative explanation for the greater net hindlimb alanine output could be that hindlimb alanine uptake is decreased because of the low circulating alanine levels after exercise. This could increase net hindlimb alanine output even if unidirectional hindlimb output is unaffected. We feel that this is unlikely because a similar reduction in alanine levels does not cause net hindlimb alanine output to be increased substantially in the absence of intraduodenal glucose. Further studies could use isotopic alanine to distinguish between effects on unidirectional alanine and output.

It could be argued that the key metabolic changes observed in the exercised dogs were an inevitable result of the postexercise state and would have occurred regardless of whether glucose was infused. This is not the case, however, because net hindlimb glutamine and alanine balances have been previously demonstrated to be constant from 10 to 90 min after the cessation of exercise in the absence of glucose infusion (19). The argument could also be made that the gradual increase in arterial alanine levels in the exercised dogs during glucose infusion may merely represent the typical postexercise response and not be dependent on glucose infusion. Again, this is not the case, because a previous

study showed that the postexercise reduction in arterial alanine still persists 90 min after the cessation of exercise (19), a time point at which the restoration of arterial alanine concentration was practically complete in the present study.

Ammonia is formed at an increased rate in working muscle and is caused by the deamination of BCAA and adenine nucleotides (5, 17). Proper muscle function requires that this free ammonia is removed. Glutamine and, to a smaller extent, alanine, are the primary vehicles for transport of ammonia out of muscle. In sedentary animals, a $44.5 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ intraduodenal glucose infusion leads to a $77.8 \mu\text{mol}/\text{min}$ increment in limb glucose uptake and no increase in net release of alanine or glutamine from skeletal muscle for at least 90 min. Limb glucose uptake in response to the intraduodenal glucose load is increased twofold more when preceded by exercise. This corresponds with an increased limb glutamine efflux, and, to a lesser extent, an increased alanine efflux. In conclusion, oral glucose may facilitate muscle recovery from prolonged exercise by enhancing the removal of ammonia in the form of glutamine and, possibly, alanine.

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