Regulation of hepatic glutamine metabolism during exercise in the dog

AMY E. HALSETH, NATHALIE RHÉAUME, ALLISON B. MESSINA, ERICA K. REED, MAHESH G. KRISHNA, PAUL J. FLAKOLL, D. BROOKS LACY, AND DAVID H. WASSERMAN
Department of Molecular Physiology and Biophysics, Diabetes Research and Training Center, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Halseth, Amy E., Nathalie Rhéaume, Allison B. Messina, Erica K. Reed, Mahesh G. Krishna, Paul J. Flakoll, D. Brooks Lacy, and David H. Wasserman. Regulation of hepatic glutamine metabolism during exercise in the dog. Am. J. Physiol. 275 (Endocrin. Metab. 38): E655–E664, 1998.—The goal of this study was to determine how liver glutamine (Gln) metabolism adapts to acute exercise in the 18-h-fasted dogs (n = 7) and in dogs that were glycogen depleted by a 42-h fast (n = 8). For this purpose, sampling (carotid artery, portal vein, and hepatic vein) and infusion (vena cava) catheters and Doppler flow probes (portal vein, hepatic artery) were implanted under general anesthesia. At least 16 days later an experiment, consisting of a 120-min equilibration period, a 30-min basal sampling period, and a 150-min exercise period was performed. At the start of the equilibration period, a constant-rate infusion of [5-15N]Gln was initiated. Arterial Gln flux was determined by isotope dilution. Gut and liver Gln release into and uptake from the blood were calculated by combining stable isotopic and arteriovenous difference methods. The results of this study show that 1) in the 18-h-fasted dog, ~10% and ~35% of the basal Gln appearance in arterial blood is due to Gln release from the gut and liver, respectively, whereas ~30% and ~25% of the basal Gln disappearance is due to removal by these tissues; 2) extending the fast to 42 h does not affect basal arterial Gln flux or the contribution of the gut to arterial Gln fluxes but decreases hepatic Gln release, causing a greater retention of gluconeogenic carbon by the liver; 3) moderate-intensity exercise increases hepatic Gln removal from the blood regardless of fast duration but does not affect the hepatic release of Gln; and 4) Gln plays an important role in channeling nitrogen into the ureagenic pathway in the basal state, and this role is increased by ~80% in response to exercise. These studies illustrate the quantitative importance of the splanchnic bed contribution to arterial Gln flux during exercise and the ability of the liver to acutely adapt to changes in metabolic requirements induced by the combined effects of fasting and exercise.

DURING PROLONGED endurance exercise, amino acid and adenosine monophosphate deamination leads to an increased formation of NH3 by skeletal muscle (45). The added NH3 formed during exercise is, in large part, shuttled to the liver (16), where it may undergo a number of fates, including ureagenesis. There is evidence that the amino acid glutamine (Gln) is an important vehicle for the interorgan transport and hepatic metabolism of nitrogen during exercise (49). In dogs (50) and humans (15), Gln release from the working limb is increased. Furthermore, studies in dogs with catheters in the portal and hepatic veins have shown that the liver is transformed by exercise from an organ that is generally a net producer of Gln to a net consumer (49). In addition to its role in nitrogen transfer, Gln is also a gluconeogenic precursor and may facilitate the maintenance of glucose homeostasis at times when demands for glucose are high and tissue glycogen stores are low (43). The liver, by virtue of the regional heterogeneity of the hepatocytes that comprise it, is capable of simultaneously producing and consuming Gln (31). It is unknown whether the effects of exercise on net hepatic Gln balance are due to a decrease in output, an increase in uptake, or both.

The purpose of these experiments was, during a bout of prolonged, moderate-intensity exercise, to establish the contribution of changes in unidirectional hepatic Gln output and uptake to changes in net hepatic Gln balance, determine the contribution of hepatic Gln output and uptake to arterial Gln appearance and disappearance, and assess whether hepatic or arterial Gln fluxes are sensitive to metabolic fuel availability (glycogen stores), and in the basal state, and this role is increased by ~80% in response to exercise. These studies illustrate the quantitative importance of the splanchnic bed contribution to arterial Gln flux during exercise and the ability of the liver to acutely adapt to changes in metabolic requirements induced by the combined effects of fasting and exercise.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

MATERIALS AND METHODS

Animal maintenance and surgical procedures. Mongrel dogs (n = 22; mean wt 22.2 ± 0.3 kg) of either gender that had been fed a standard diet (Kal Kan beef dinner, Vernon, CA, and Wayne Lab Blox: 51% carbohydrate, 31% protein, 11% fat, and 7% fiber based on dry wt, Allied Mills, Chicago, IL) were studied. Dogs were fed once daily. The total energy intake of this diet corresponded to 85 kcal·kg⁻¹·day⁻¹, and the absolute protein intake was 6 g·kg⁻¹·day⁻¹. Body weight was unchanged from the day of surgery to the day of the experiment in dogs maintained on this diet. The dogs were housed in a facility that met American Association for Accreditation of Laboratory Animal Care guidelines, and the protocol was approved by the Institutional Animal Care and Use Committee of Vanderbilt University School of Medicine. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
hearts were approved by Vanderbilt University's Institutional Animal Care and Use Subcommittee. At least 16 days before each experiment, a laparotomy was performed under general anesthesia (0.04 mg/kg of atropine and 15 mg/kg pentothal sodium presurgery and 1.0% isoflurane inhalation anesthetic during surgery). Silastic catheters (0.03 in. ID) were inserted into the vena cava for infusions. 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 for the placement of a sampling catheter in the carotid artery. The carotid artery was isolated, and a Silastic catheter (0.04-in. ID) was inserted so that its tip rested in the aortic arch. After insertion, the catheters 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 Univ. School of Medicine) were used to measure portal vein and hepatic artery blood flows (26, 30). Briefly, a small section of the portal vein, upstream from its junction with the gastroduodenal vein, was cleared of tissue, and a 7.0-mm-inside diameter flow 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 3.0-mm-inside diameter flow cuff was placed around the vessel and secured. The Doppler probe leads and the knotted free catheter ends, with the exception of the knotted end of the carotid artery catheter, were stored in a subcutaneous pocket in the abdominal region so that complete closure of the skin incision was possible. The free end of the carotid artery catheter was stored under the skin of the neck.

Starting 1 wk after surgery, 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 1) a leukocyte count <18,000/mm³, 2) a hematocrit >36%, 3) normal stools, and 4) 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 under local anesthesia (2% lidocaine; Astra Pharmaceutical Products, Worcester, MA) on the subcutaneous pockets in which catheters were stored. The contents of each catheter were aspirated, and 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 was infused in the arterial catheter throughout experiments (0.1 ml/min).

Experimental procedures. Experiments consisted of a 120-min equilibration period (150 to 30 min), a 30-min basal sampling period (30 to 0 min) and a 150-min moderate-intensity exercise period (0 to 150 min). The dogs were subdivided into those in which the equilibration period was started after an 18-h (n = 7) or a 42-h (n = 8) fast. Estimates of hepatic glycojen content and splanchnic lactate uptake in dogs and humans suggest that the 18- and 42-h-fasted dogs (13, 14) are in absorptive states similar to humans (2, 17, 35) that are ~8 h and 16 h fasted, respectively. Exercise was performed at 100 ml/min with a 12% grade on a motorized treadmill. The exercise intensity used in these experiments has previously been shown to result in a twofold increase in heart rate and an increase in O₂ uptake to 50% of maximum (41). A group consisting of 7 dogs was studied as described above, except that the dogs remained sedentary from time (t) = 0 to t = 150 min. After a baseline arterial blood sample was obtained, a primed constant-rate venous infusion of [5-¹³C]Gln (36 µmol/kg prime, 0.4 µmol · kg⁻¹·min⁻¹ infusion) was initiated at t = -150 min in all dogs studied, as was an infusion of indocyanine green (ICG; 0.1 mg·m⁻²·min⁻¹). A primed constant-rate infusion of [1-¹³C]leucine was also given at this time. Data pertaining to isotopic leucine have been published (20). Stable isotopes were obtained from Cambridge Isotope Laboratories (Andover, MA), and ICG was purchased from Hynson, Westcott, and Dunning (Baltimore, MD). ICG was used as an independent backup measurement of splanchnic blood flow and as a means of confirming hepatic vein catheter placement. Good agreement for flow rates obtained with Doppler flow probes and ICG measurements has previously been demonstrated (22). Arterial, portal venous, and hepatic venous samples were drawn at t = -30, -15, 0, 25, 37.5, 50, 75, 87.5, 100, 125, 137.5, and 150 min. Portal vein and hepatic artery blood flows were recorded continuously on-line (26).

Processing of blood and tissuesamples. Blood samples were collected in heparinized syringes and placed in tubes containing 1) EDTA for analysis of plasma, 2) 4% perchloric acid for analysis of whole blood, or 3) EGTA and glutathione for analysis of plasma catecholamines. Samples were then centrifuged, and the supernatant was removed. Gln (34) and urea (33) concentrations were measured in the supernatant from perchloric acid-deproteinized blood. Plasma Gln enrichment, expressed as the molar percent excess, was determined using the tertiary butyldimethylsilyl derivatives. Derivatized samples were analyzed on a Hewlett-Packard 5975 gas chromatography-mass spectrometry system with the use of electron ionization and selected ion monitoring of ions with mass-to-charge ratios of 431/432 for Gln. Plasma urea enrichment was measured by Metabolic Solutions (Merrimack, NH). For this purpose, samples were reacted with urease in a sealed tube in which a trapping well containing H₂SO₄ was placed. The NH₄ that was formed was released from solution using an alkalinizing agent (5 M NaOH) and trapped in the well forming ammonium sulfate. The nitrogen enrichment of an aliquot of the ammonium sulfate solution was analyzed with the use of ion ratio mass spectrometry with the ANCA combustion preparation system (Europa Scientific). Plasma insulin, glucagon, cortisol, and catecholamine analyses were done by established techniques that have been described previously (22).

Calculations. Rates of Gln appearance (R_a) in and disappearance (R_d) from arterial plasma were measured using an isotope dilution method described by Eqs. 1 and 2, respectively. The validity of this method has been recently assessed for the Gln system (32)

\[ R_a = \frac{(R^*E_a)}{-p} - \frac{p \times V \times [Gln]_a \times dE_a/dt \times E_a}{E_a} \]  

\[ R_d = R_a - \frac{p \times V \times d[Gln]_a/dt}{E_a} \]

where \( R^* \) is the infusion rate of [5-¹³N]Gln, \( E_a \) is the arterial enrichment of Gln, \([Gln]_a \) refers to the blood Gln concentrations, subscript a is used to designate arterial blood, V is the volume of Gln distribution, and p is the pool fraction. V and p are assumed to be 360 ml/kg and 0.75, respectively, based on the work of Kreider et al. (32).

Net gut and liver Gln balance (NGGlnB and NHGlnB, respectively) were calculated as follows

\[ NGGlnB = ([Gln]_a - [Gln]_p) \times BF_{pv} \]

\[ NHGlnB = [Gln]_p \times BF_{pv} + [Gln]_a \times BF_{ha} - [Gln]_a \times BF_{ha} \]
where the subscripts pv, ha, and hv refer to portal vein, hepatic artery, and hepatic vein, respectively, and BF refers to blood flow. Net hepatic urea output was calculated by an equation analogous to Eq. 4, except that the sign (+/−) was reversed so that positive numbers reflect net output. Unidirectional gut (GGlnFE) and hepatic fractional Gln extraction (HGlnFE) values were calculated with the use of isotopic Gln such that

\[
GGlnFE = \left( \frac{E_a \times [Gln]_a - E_{pv} \times [Gln]_{pv}}{(E_a \times [Gln]_a) / (E_a \times [Gln]_a)} \right) E657
\]

\[
HGlnFE = \left( \frac{E_{pv} \times [Gln]_{pv} \times BF_{pv} + E_a \times [Gln]_a \times BF_{ha}}{(E_{pv} \times [Gln]_{pv} \times BF_{pv} + E_a \times [Gln]_a \times BF_{ha})} \right) / (E_{pv} \times [Gln]_{pv} \times BF_{pv} + E_a \times [Gln]_a \times BF_{ha}) \]

Gln delivery to gut and liver (GGlnD and HGlnD, respectively) was calculated by the following equations

\[
GGlnD = [Gln]_a \times BF_{pv}
\]

\[
HGlnD = [Gln]_a \times BF_{ha} + [Gln]_{pv} \times BF_{pv}
\]

Unidirectional gut (GGlnU) and hepatic Gln uptake (HGlnU) from the blood could then be calculated from the preceding equations

\[
GGlnU = GGlnFE \times GGlnD
\]

\[
HGlnU = HGlnFE \times HGlnD
\]

and unidirectional gut (GGlnO) and hepatic Gln output (HGlnO) into the blood could be calculated as

\[
GGlnO = GGlnU - NGGlnB
\]

\[
HGlnO = HGlnU - NHGlnB
\]

The rate of conversion of the Gln amide nitrogen into urea was calculated as described below

\[
\Delta Urea E_a = 150 - 0 min/p.
\]

\[
\int_0^{150} Gln E_{hv} \times V \times [urea]_a dt
\]

where \( t = 150 \) min, \( V \) is the volume of urea distribution in the whole body and is assumed to be 600 ml/kg (37), \( \Delta \) represents change, and \( [urea]_a \) is urea concentration in arterial blood.

Data analysis. Experiments consisted of four sampling periods. These were the 30-min basal period and the 25- to 50-min, the 75- to 100-min, and the 125- to 150-min exercise periods. Sample points (listed in Experimental Procedures) were presented individually or pooled with other sample points in the sampling period. Statistics were performed using SuperAnova (Abacus Concepts, Berkeley, CA) on a Macintosh Power personal computer. Statistical comparisons between groups and over time were made using ANOVA designed to account for repeated measures. Significant differences were assessed using contrasts solved by univariate repeated measures. Differences are considered significant if \( P < 0.05 \). Data are expressed as means ± SE.

RESULTS

Arterial plasma hormone concentrations and portal vein and hepatic artery blood flows. Arterial plasma insulin, glucagon, catecholamine, and cortisol concentrations and portal vein and hepatic artery blood flows from these studies have been published previously (20) and are only summarized. Unless otherwise stated, exercise values represent those obtained between \( t = 125 \) and 150 min. Plasma glucagon rose from basal values of 45 ± 9 and 38 ± 6 pg/ml to 82 ± 28 and 115 ± 46 pg/ml during exercise in 18- and 42-h-fasted dogs, respectively (\( P < 0.05 \)). Plasma insulin fell from 9 ± 3 and 6 ± 1 µU/ml in the basal state to 3 ± 0.4 and 6 ± 2 µU/ml with exercise in 18- and 42-h-fasted dogs, respectively (\( P < 0.05 \)). Plasma cortisol increased from 1.7 ± 0.2 and 2.6 ± 0.4 µg/dl in the basal state to 7.0 ± 2.1 and 9.3 ± 2.4 µg/dl during exercise in 18- and 42-h-fasted dogs, respectively (\( P < 0.05–0.01 \)). Basal cortisol was significantly elevated in 42- compared with 18-h-fasted dogs. Cortisol levels at \( t = 25–50 \) min of exercise were less (\( P < 0.05 \)) in 18-h-fasted dogs (1.6 ± 0.3 µg/dl) than in 42-h-fasted dogs (5.6 ± 1.2 µg/dl). Plasma norepinephrine increased from 149 ± 27 and 189 ± 27 pg/ml in the basal state to 430 ± 48 and 1,103 ± 268 pg/ml during exercise in 18- and 42-h-fasted dogs, respectively (\( P < 0.01 \)). Plasma epinephrine increased from 67 ± 28 and 75 ± 25 pg/ml in the basal state to 194 ± 34 and 895 ± 316 pg/ml during the last 25 min of exercise in 18- and 42-h-fasted dogs, respectively (\( P < 0.01 \)). Exercise-induced increases in norepinephrine and epinephrine were both greater during the 42-h fast. Hormone levels were unchanged throughout the experiment in 18-h-fasted dogs that remained sedentary.

Arterial Gln concentrations and kinetics. Arterial Gln concentration fell gradually, by 10–20%, during exercise in both 18- and 42-h-fasted dogs (Fig. 1). Arterial Gln concentrations have been reported to range from ~600 to ~850 µM in the conscious dog (1, 7–10, 19, 49). Arterial basal Gln concentration was within this range and was unchanged over time in 18-h-fasted dogs that remained sedentary for the duration of the study (Table 1). \( R_a \) and \( R_d \) were similar in the basal period in 18- and 42-h-fasted dogs (Fig. 1). Exercise did not significantly alter arterial Gln kinetics. In the sedentary dogs, however, Gln kinetics actually fell slightly so that \( R_a \) and \( R_d \) were reduced by ~20% in the last sampling interval compared with the first (Table 1). Thus, compared with sedentary controls, exercise resulted in a small increase in arterial Gln flux in both 18- and 42-h-fasted dogs.

Gut Gln kinetics. The gut was a net consumer of Gln in the basal state, and fast duration and exercise did not significantly affect this variable. Gut Gln output was ~5–10% of the Gln \( R_a \) in the basal state in both 18-
and 42-h-fasted dogs (Tables 1 and 2). Gut Gln output was not significantly affected by exercise in either 18- or 42-h-fasted dogs. Gut Gln uptake comprised 20–30% of Rd in resting 18-h-fasted dogs. Gut Gln uptake was unaffected by exercise in 18-h-fasted dogs but increased transiently in 42-h-fasted dogs (P < 0.05 at t = 25–50 min). By the end of exercise, gut Gln uptake was actually lower in 42-h-fasted compared with 18-h-fasted dogs. Approximately 10–20% of the Gln delivered to these tissues was extracted in the basal state. Basal hepatic fractional Gln extraction was not different in dogs fasted for 18 and 42 h. This variable was increased by approximately twofold during exercise compared with the basal period (−30 to 0 min) but not compared with the increase that was evident in the sedentary control group.

Gut Gln output and uptake did not change significantly in 18-h-fasted sedentary control dogs over the duration of the experiment (Table 1). Gut Gln fractional extraction, however, was significantly elevated at t = 125–150 min, attaining a value equivalent to that seen during exercise at the same time interval in the 18-h-fasted dogs (P < 0.05). It is unclear why gut fractional Gln extraction is increased in the last time period or whether this change is physiologically important.

Hepatic Gln kinetics. In 18-h-fasted dogs, the liver went from being a net producer of Gln in the basal state to being a significant net consumer throughout exercise (P < 0.005–0.05; Fig. 2). In 42-h-fasted dogs, net hepatic Gln balance was essentially "0" at rest (Fig. 2). As was the case in 18-h-fasted dogs, the liver became a significant net consumer of Gln during exercise in...
42-h-fasted dogs ($P < 0.01$–$0.005$). Net hepatic Gln balance was higher in 42-h-fasted dogs compared with 18-h-fasted dogs in the basal state and in the first two exercise periods ($P$, $0.05$–$0.01$). This difference in net hepatic Gln balance was due to a marked reduction in hepatic Gln output. Increasing the fast duration to 42 h significantly reduced hepatic Gln output to $25\%$ of the rate in 18-h-fasted dogs in the basal state and throughout the exercise period ($P$, $0.005$). The increased net hepatic uptake of Gln that occurred in response to exercise in 18- and 42-h-fasted dogs was due to significant increases in hepatic Gln uptake ($P$, $0.01$ and $0.005$ at $75$–$100$ min and $125$–$150$ min in 18-h-fasted dogs and $P < 0.05$ from $t = 75$ to $100$ min in 42-h-fasted dogs; Fig. 2), as hepatic Gln output was unchanged. Exercise increased hepatic Gln uptake to a higher rate in 18- compared with 42-h-fasted dogs ($P$, $0.05$ and $0.005$ at $t = 75$–$100$ min and $t = 125$–$150$ min). The effects of exercise on hepatic Gln uptake were a result of increases in hepatic Gln fractional extraction in both 18- and 42-h-fasted dogs ($P$, $0.005$; Fig. 3). The increase in hepatic Gln fractional extraction with exercise, as with the increase in hepatic Gln uptake, was significantly attenuated by the extended fast duration, as values were reduced by $30\%$ in 42- compared with 18-h-fasted dogs ($P < 0.05$).

### Table 2. Gut Gln net balance, unidirectional output and uptake, and fractional extraction during rest and exercise in 18- and 42-h-fasted dogs

<table>
<thead>
<tr>
<th></th>
<th>Basal (−30 to 0 min)</th>
<th>25–50 min</th>
<th>75–100 min</th>
<th>125–150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Net gut Gln balance, µmol·kg$^{-1}$·min$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>$1.6 \pm 0.6$</td>
<td>$1.7 \pm 0.4$</td>
<td>$1.4 \pm 0.5$</td>
<td>$1.5 \pm 0.5$</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>$1.5 \pm 0.2$</td>
<td>$1.6 \pm 0.5$</td>
<td>$0.9 \pm 0.2$</td>
<td>$0.7 \pm 0.3$</td>
</tr>
<tr>
<td><strong>Gut Gln output, µmol·kg$^{-1}$·min$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>$1.0 \pm 0.7$</td>
<td>$0.9 \pm 0.4$</td>
<td>$1.3 \pm 0.5$</td>
<td>$1.5 \pm 0.3$</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>$0.6 \pm 0.5$</td>
<td>$1.6 \pm 0.5$</td>
<td>$0.8 \pm 0.4$</td>
<td>$0.8 \pm 0.3$</td>
</tr>
<tr>
<td><strong>Gut Gln uptake, µmol·kg$^{-1}$·min$^{-1}$</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>$2.6 \pm 0.5$</td>
<td>$2.6 \pm 0.5$</td>
<td>$2.8 \pm 0.7$</td>
<td>$3.1 \pm 0.4$</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>$2.0 \pm 0.5$</td>
<td>$3.2 \pm 0.7^*$</td>
<td>$1.7 \pm 0.5$</td>
<td>$1.5 \pm 0.4^*$</td>
</tr>
<tr>
<td><strong>Gut fractional Gln extraction</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>$0.13 \pm 0.02$</td>
<td>$0.18 \pm 0.05$</td>
<td>$0.20 \pm 0.06^*$</td>
<td>$0.24 \pm 0.03^*$</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>$0.13 \pm 0.03$</td>
<td>$0.23 \pm 0.03^*$</td>
<td>$0.16 \pm 0.05$</td>
<td>$0.16 \pm 0.03$</td>
</tr>
</tbody>
</table>

Data are means ± SE; $n = 7$ and 8 dogs that were 18 and 42 h fasted, respectively. A positive net balance reflects net uptake. *Significantly different from basal, $P < 0.05$–$0.02$. †Significantly different from corresponding value in 18-h-fasted dogs, $P < 0.002$. 

Fig. 2. Effect of 150 min of treadmill exercise on net hepatic Gln balance, hepatic Gln output, and hepatic Gln uptake in 18- (A) and 42-h-fasted dogs (B). Data are means ± SE calculated from average of 3 measurements spanning each interval. *Significantly different from basal values ($P < 0.05$–$0.005$); †significantly different from values in 18-h-fasted dogs ($P < 0.05$–$0.005$); $n = 7$ for 18- and $n = 8$ for 42-h-fasted dogs.
Hepatic net balance, output, uptake, and fractional extraction of Gln were constant in dogs remaining sedentary throughout the experiment (Table 1).

Arterial blood glutamate concentrations, net gut balances, and net hepatic balances. There was a gradual increase in arterial glutamate (Glu) concentrations in both 18- and 42-h-fasted dogs in response to exercise (Table 3; P < 0.05). The gut was a net producer of Glu during rest in 18- and 42-h-fasted dogs. Net gut Glu release tended to increase and decrease during exercise in 18- and 42-h-fasted dogs, respectively. Neither change was significant. However, in parallel with the lower net gut Gln uptake at the end of exercise in 42-h-fasted dogs compared with 18-h-fasted dogs, the net gut release of Gln was also lower (P < 0.05). In the basal state, net hepatic balance of Glu and net hepatic Glu fractional extraction were not different from 0. In response to exercise, there was a gradual increase in net uptake and net fractional extraction in 18-h-fasted dogs. These variables were unaffected by exercise in 42-h-fasted dogs.

Arterial plasma urea concentrations, net output, and conversion from Gln. Arterial urea levels were similar in 18- and 42-h-fasted dogs (Fig. 4). Both groups exhibited small but significant increases in urea levels with exercise (P < 0.05–0.001 at t = 75–100 min and t = 125–150 min). Arterial urea levels also rose slightly, however, in dogs that remained sedentary for the duration of the study (Table 4). Net hepatic urea output was also unaffected by fast duration (Fig. 4). Exercise resulted in significant increases in net hepatic urea output that were similar in 18- and 42-h-fasted dogs (P < 0.05–0.01 at t = 75–100 min and t = 125–150 min). Net hepatic urea output was unchanged in dogs that remained sedentary (Table 4).

To assess the role of Gln in nitrogen transport to the liver during exercise, the enrichment of urea nitrogen was measured in arterial samples of 18-h-fasted sedentary and exercised dogs (Fig. 5). Hepatic conversion of Gln to urea was increased by ~80% by exercise (P < 0.05). From the data in Table 1 and Figs. 2A and 5, it could be calculated that more of the Gln taken up by the liver was channeled into urea during exercise (117 ± 12%) compared with rest (74 ± 18%).

**DISCUSSION**

This study shows that the basal R_{a} and R_{b} in the dog are ~10 µmol·kg⁻¹·min⁻¹. It can be estimated from the results of these studies that ~10% of the basal R_{a} is due to gut Gln output and ~35% is due to hepatic Gln output in 18-h-fasted dogs. In 42-h-fasted dogs, basal R_{a} and the gut contribution to basal R_{b} are essentially the same as in 18-h-fasted dogs. The contribution of the

---

**Table 3.** Arterial concentrations, net gut balances, and net hepatic balances of glutamate during rest and exercise in 18- and 42-h-fasted dogs

<table>
<thead>
<tr>
<th></th>
<th>Basal -30 to 0 min</th>
<th>Exercise 25-50 min</th>
<th>Exercise 75-100 min</th>
<th>Exercise 125-150 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial concentration, µM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>72 ± 8</td>
<td>81 ± 7</td>
<td>87 ± 10*</td>
<td>94 ± 11*</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>96 ± 18</td>
<td>105 ± 20</td>
<td>123 ± 21*</td>
<td>125 ± 16*</td>
</tr>
<tr>
<td>Net gut balance, µmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>-0.17 ± 0.03</td>
<td>-0.24 ± 0.11</td>
<td>-0.25 ± 0.07</td>
<td>-0.32 ± 0.15</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>-0.14 ± 0.03</td>
<td>-0.19 ± 0.05</td>
<td>-0.04 ± 0.14††</td>
<td>-0.07 ± 0.11††</td>
</tr>
<tr>
<td>Net hepatic balance, µmol·kg⁻¹·min⁻¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>-0.03 ± 0.09</td>
<td>0.15 ± 0.09</td>
<td>0.17 ± 0.07*</td>
<td>0.29 ± 0.09*</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>0.02 ± 0.04</td>
<td>-0.06 ± 0.15</td>
<td>0.12 ± 0.10</td>
<td>-0.06 ± 0.04††</td>
</tr>
<tr>
<td>Net hepatic fractional extraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 h Fasted</td>
<td>0.02 ± 0.06</td>
<td>0.06 ± 0.05</td>
<td>0.08 ± 0.04</td>
<td>0.11 ± 0.03*</td>
</tr>
<tr>
<td>42 h Fasted</td>
<td>0.00 ± 0.02</td>
<td>0.04 ± 0.03</td>
<td>0.05 ± 0.02</td>
<td>0.00 ± 0.02††</td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7 and 8 dogs that were 18 and 42 h fasted, respectively. A positive net balance reflects net uptake. *Significantly different from basal, P < 0.05–0.01. †Significantly different from corresponding value in 18-h-fasted dogs, P < 0.05–0.02.
liver, however, is reduced to <10% with the extended fast. It can be approximated that the contribution of total splanchnic Gln output to total flux is reduced in 42-h-fasted dogs by approximately two-thirds compared with 18-h-fasted dogs. Although hepatic Gln output is reduced in the 42-h-fasted dog compared with the 18-h-fasted dog, arterial Gln kinetics are not. This suggests that Gln output at one or more other sites is accelerated. The probable extrasplanchnic source of the increased Gln released into the blood is likely to be the skeletal muscle (5, 42).

The contributions of both gut and liver Gln uptake to basal R_{d} are considerable. The gut is responsible for ~30% of the Gln consumed in the basal state, and the liver is responsible for ~25% in the 18-h-fasted dogs. Basal R_{d} in 42-h-fasted dogs is similar to that seen in 18-h-fasted dogs. In 42-h-fasted dogs, the contributions of the gut and liver Gln uptake to basal R_{d} are ~20 and 10%. It is surprising that although the liver is more gluconeogenic after the extended fast (11), the contribution of the liver to Gln removal was not greater. Nevertheless, one consequence of the marked reduction in hepatic Gln output in 42-h-fasted dogs is that, in a net sense, more Gln is conserved in the liver potentially for use as a gluconeogenic substrate.

Table 4. Arterial urea concentration and net hepatic urea output in 18-h-fasted dogs sedentary for study duration

<table>
<thead>
<tr>
<th>Arterial urea concn, mM</th>
<th>NHUreaO, µmol·kg⁻¹·min⁻¹</th>
<th>Basal</th>
<th>Sedentary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal – 30 to 0 min</td>
<td>4.1 ± 0.5*</td>
<td>4.2 ± 0.5*</td>
<td>4.2 ± 0.6*</td>
</tr>
<tr>
<td>25 to 50 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 to 100 min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125 to 150 min</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SE; n = 7 18-h-fasted sedentary dogs. NHUreaO, net hepatic output of urea. *Significantly different from basal values, P < 0.02–0.005.

The greater net hepatic Gln uptake during exercise was due to a two- to threefold stimulation of hepatic uptake and fractional extraction of Gln. The signal causing the increase may be hormonal. Exercise increased cortisol by approximately threefold. Data from the anesthetized, adrenalectomized rat showed that chronic glucocorticoid replacement to levels seen during trauma increased hepatic Gln uptake (46). This increase corresponded to an elevation in hepatic system nitrogen transport and glutaminase activities. It is unknown whether the changes in cortisol evident with exercise contribute to changes in hepatic Gln metabolism, especially within the exercise period. Another mediator of this response may be glucagon. Glucagon stimulates net Gln uptake by the isolated, perfused rat liver and increases glutaminase activity (3) and flux through the glutaminase step (27). An increase in portal vein ammonia can serve as a feed-forward signal by increasing hepatic glutaminase activity (4, 8, 28).
The exercising dog exhibits a small transient increase in portal vein ammonia (48). It seems unlikely that this would result in hepatic concentrations that could lead to a sustained increase in glutaminase. An increase in gut Gln fractional extraction with exercise was seen in both 18- and 42-h-fasted dogs. The 18-h-fasted sedentary dogs exhibited an increase in gut Gln fractional extraction at the 125- to 150-min sample interval. Gut Gln uptake also tended to increase at this last sample interval, but this change was not significant. Because sedentary animals undergoing an extended period of rest exhibit a response that is similar to the response during exercise, it cannot be concluded that changes at the gut are due to the exercise bout per se.

Exercise results in an increase in gut proteolysis (20, 55) and net release of α-amino nitrogen (49) in the dog. With the increase in gut proteolysis during exercise, one might predict an increase in unidirectional Gln release during exercise. The fact that this was not observed reflects a limitation of the tracer method. This limitation results because Gln may be produced and consumed in the same tissue without entering or equilibrating with the blood. As a consequence, unidirectional balances reflect the flux of Gln into and out of the blood. This method is useful in assessing interorgan exchange but only estimates metabolic production and utilization within the tissues.

The measurement of arterial Gln flux indicated that this variable was unchanged with exercise, whereas it fell by ~15% in the 18-h-fasted sedentary dogs. Thus Gln flux in exercised animals was increased compared with that in sedentary animals. One could speculate that the [5-15N]Gln is recycled as the result of an increase in secondary labeling of NH₃ pools as study duration is increased. If this is the case, Gln flux could be underestimated, resulting in an apparent reduction in Gln flux in the sedentary animals and the masking of an increase in the exercised animals. An increase in Gln flux is consistent with the liver removing more Gln from the blood and the earlier demonstration that the hindlimb is contributing more Gln to the blood (50).

Renal Gln metabolism is an important component of the defense against acidosis, with an increase in Gln utilization and ammonia production by the kidney when pH is decreased (52, 53). Arterial pH in dogs (56), like humans (51), does not decrease in response to moderate-intensity exercise. Therefore, one would not predict an increase in renal Gln metabolism on this basis.

In contrast to the response of arterial Gln, which fell gradually with exercise, arterial Glu rose regardless of the fast duration. Moreover, it did so in the 18-h-fasted dogs despite an increase in net hepatic uptake and fractional extraction of Glu. Although the extended fast did not significantly affect the response of arterial Glu to exercise, it alleviated the increase in net hepatic uptake and fractional extraction of Glu. The absence of an increase in net hepatic Glu uptake with exercise in 42-h-fasted animals does not fit with a greater reliance on gluconeogenic precursors and gluconeogenesis in these animals. There was a greater net release of Glu from the gut in 18-h-fasted dogs compared with 42-h-fasted dogs. Because the hepatic fractional extraction of Glu (as well as some other amino acids) is sensitive to changes in portal vein delivery (40), it may be that the resulting increase in portal vein Glu in 18-h-fasted dogs was responsible for the increase in the extraction of this amino acid by the liver.

Basal blood Gln flux was ~10 µmol·kg⁻¹·min⁻¹ in 18- and 42-h-fasted dogs, which is approximately twofold the basal rate in postabsorptive humans (12, 23, 24, 43, 54). This difference is sustained during exercise (54). The higher Gln flux is consistent with the higher leucine flux and proteolysis in dogs (20, 29, 47). Previous studies have shown, using arteriovenous differences, that the splanchnic bed of human subjects is a net consumer of Gln (18, 36). Studies using isotopic Gln have demonstrated the high capacity of the human splanchnic bed to utilize Gln (38). By summing net hepatic and gut Gln balances, one can see that net splanchnic Gln uptake is also present in dogs. It has been shown previously that in the basal state, the gut is responsible for all the net uptake of Gln, whereas during exercise, the net uptake of Gln by the liver can exceed that of the gut (49). The contribution of the gut and liver to net splanchnic Gln uptake is impossible to assess in conscious humans. It has been shown in overnight-fasted patients, in which portal vein blood was sampled during surgery, that the gastrointestinal tract, and not the liver, is the main site of splanchnic Gln removal (18). Furthermore, portal vein Gln was approximately equal to hepatic vein Gln (18). This suggests that net hepatic Gln uptake is small and possibly negligible in overnight-fasted humans. The manner in which human hepatic Gln metabolism adapts to exercise has not been assessed.

This study confirms that exercise increases net hepatic urea output in the dog (49). This contrasts with work in humans showing that isotope-determined urea production was unchanged during 3 h of light exercise and 1 h of heavy exercise (6). The reason for this discrepancy may relate to difficulties in measuring acute increases in urea output isotopically (21), since other indexes of urea production (i.e., urea excretion, blood levels, sweat loss) can increase with exercise (25, 44). The increase in net hepatic urea output was paralleled by the increase in hepatic Gln uptake. In addition, there was an ~80% increase in the rate at which the amide nitrogen of Gln was incorporated into urea with exercise. It can be estimated that essentially all of the amide nitrogen on the Gln taken up by the liver went to the formation of urea during rest and exercise. This is consistent with data from hepatocytes showing that changes in [15N]urea synthesis from [5-15N]Gln correspond to changes in flux through glutaminase (3). One could calculate that a considerable percentage of the total urea nitrogen was derived from Gln (a range of ~30–60%). However, only a minor percentage of the exercise-induced increment in net hepatic urea output could be derived from the Gln amide nitrogen. In 18-h-fasted dogs, net hepatic urea output rose by ~5 µmol·kg⁻¹·min⁻¹ during exercise.
The exercise-induced rise in the rate at which urea is formed from the Gln amide nitrogen was \(~1.2\ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\). Therefore, \(~25\%\) of the increase in urea release from the liver is derived from the Gln amide nitrogen. Net hepatic \(\alpha\)-amino nitrogen uptake increases by \(~10–15\ \mu\text{mol}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}\) during treadmill exercise of the same duration and intensity used in these studies (49). This could more than account for the formation of urea during exercise.

It is likely that the increase in net hepatic urea output is driven, in part, by the increased extraction of Gln and other amino acids by the liver with exercise. This is consistent with the demonstration that an infusion of Gln that leads to an increase in net hepatic Gln uptake stimulates net hepatic urea output in the dog (10). The increase in glucagon may also facilitate the conversion of the Gln amide nitrogen to urea. Glucagon stimulates net hepatic Gln uptake and ureagenesis in perfused rat liver (27) and increases the incorporation of the Gln amide nitrogen into urea in isolated rat hepatocytes (3). The mechanism for the glucagon-stimulated increase in urea formation appears to relate to an increase in glutaminase activity. Flux through the glutaminase reaction is closely coupled to the activity of the key ureagenic enzyme, carbamoyl-phosphate synthetase (3). This link occurs because the glutaminase reaction results in the formation of N-acetylglutamate and ammonia within the mitochondria, thereby increasing the effective concentration of these compounds that activate carbamoyl-phosphate synthetase. The tight link between the fluxes through the glutaminase pathway and carbamoyl-phosphate synthetase is consistent with evidence that the Gln amide nitrogen is used preferentially to other nitrogen sources for urea synthesis (39).

In summary, the results of this study show that 1) in the 18-h-fasted dog, \(~10\%\) and \(~35\%\) of the basal arterial Gln appearance are due to Gln release from the gut and liver, respectively, whereas \(~30\%\) and \(~25\%\) of the basal Gln disappearance are due to removal by these tissues; 2) extending the fast to 42 h does not affect basal arterial Gln flux or the contribution of the gut to arterial Gln fluxes but decreases hepatic Gln release; 3) moderate exercise increases hepatic Gln removal from the blood regardless of fast duration but does not affect the hepatic release of Gln; and 4) Gln plays an important role in channeling nitrogen into the ureagenic pathway in the basal state, and this role is increased by \(~80\%\) in response to exercise. These studies illustrate the importance of the splanchnic bed contribution to arterial Gln flux and the ability of the liver to acutely adapt to changes in metabolic requirements induced by exercise.

We are grateful to Li Zheng, Wanda Snead, Pamela Venson, Eric Allen, Rob Allison, and Thomas Becker for excellent technical assistance.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant RO1-DK-47344 and Diabetes Research and Training Center Grant 5-P60-DK-20593.

Address for reprint requests: A. E. Halseth, Light Hall Rm. 702, Dept. of Molecular Physiology and Biophysics, Vanderbilt Univ. School of Medicine, Nashville, TN 37232-0615.

Received 22 January 1998; accepted in final form 13 July 1998.

REFERENCES