Metabolic Fate of Extrahepatic Arginine in Liver After Burn Injury

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Increased nitrogen loss in the form of urea is a hallmark of the metabolic aberrations that occur after burn injury. As the immediate precursor for urea production is arginine, we have conducted an investigation on the metabolic fate of arginine in the liver to shed light on the metabolic characteristics of this increased nitrogen loss. Livers from 25% total surface burn (n = 8) and sham burn rats (n = 8) were perfused in a recycling fashion with a medium containing amino acids and stable isotope labeled L-[$^{15}N_2$-guanidino, 5,5-$^2H_2$]arginine for 120 minutes. The rates of glucose and urea production and oxygen consumption were measured. The rate of unidirectional arginine transport and the intrahepatic metabolic fate of arginine in relation to urea cycle activity were quantified by tracing the disappearance rate of the arginine tracer from and the appearance rate of [$^{15}N_2$]urea in the perfusion medium. Perfused livers from burned rats showed higher rates of total urea production (mean ± SE, 4.471 ± 0.274 *v* 3.235 ± 0.261 μmol · g dry liver^{-1} · min^{-1}; *P* < .01). This was accompanied by increased hepatic arginine transport (1.269 ± 0.263 *v* 0.365 ± 0.021 μmol · g dry liver^{-1} · min^{-1}) and an increased portion of urea production from the transported extrahepatic arginine (12.9% ± 2.9% *v* 3.5% ± 0.4%, *P* < .05). The disposal of arginine via nonurea pathways was also increased (0.702 ± 0.185 *v* 0.257 ± 0.025 μmol/g dry weight^{-1}/min^{-1}; *P* < .05). We propose that increased inward transport and utilization of extrahepatic arginine by the liver contributes to the accelerated urea production after burn injury and accounts, in part, for its conditional essentiality in the nutritional support of burn patients.
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HYPERMETABOLISM and significantly elevated net nitrogen loss are hallmarks of the metabolic aberrations commonly seen in severely burned patients. Because more than 90% of nitrogen loss from these patients is in the form of urea,[1] exploring the regulation of urea cycle activity in this catabolic condition might shed light on the mechanism(s) underlying the accelerated rate of urea production. Our previous study[2] showed that when perfused with a constant medium composition, livers from burned rats produced more urea than those from sham burn rats. It appears that urea cycle activity is intrinsically upregulated in response to burn-induced stress, but the contributing metabolic factors remain to be determined. Arginine is an intermediate of the urea cycle, serving as the immediate precursor for urea synthesis. The amino acid is present in the circulatory system and can also be synthesized de novo within the kidney and liver, where in the latter case, the nitrogen source is derived from other amino acids through a spatially organized urea cycle enzyme system.[3] The source of the arginine responsible for the elevated rate of hepatic urea production remains unclear, as does the major metabolic fate of extrahepatic arginine in the burn injury-induced protein catabolic state. In burn patients, the turnover of arginine in the peripheral compartment is increased, and we have concluded that arginine is an indispensable amino acid in the nutrition of these patients.[4-7] However, the underlying metabolic basis for a dietary requirement for arginine in severe stress remains to be fully understood.

The transport of arginine into the liver has been studied using hepatocyte membrane vesicle preparations. Pacitti et al[8] reported a significantly activated y^+ system, responsible mainly for arginine transport. These investigators observed increased transport kinetics of arginine by hepatic membrane vesicles in rats with septicemia, indicating that a septic condition promotes utilization of extrahepatic arginine by the liver. However, the intrahepatic metabolic fate of arginine cannot be elucidated with vesicle preparations. The present study used an in situ liver perfusion system combined with the use of a L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine tracer. The rate of arginine transport and its major rates of intrahepatic metabolism in relation to the activated urea cycle after burn injury were evaluated. These studies show an increased consumption of preformed arginine from extrahepatic sources for urea production. Our findings help to further interpret the results of clinical investigations of arginine metabolism in severely burned patients.[4,5]

MATERIALS AND METHODS

Burn and Sham Burn Animal Model

The studies were performed using 16 male Sprague-Dawley rats (Charles River Laboratories, Boston, MA) weighing 220 to 280 g. Rats were housed in the Animal Farm of the Massachusetts General Hospital under 12-hour light-dark cycle for at least 2 days before the study took place. The animals were cared for in accordance with the Public Health Service Policy, the Guideline for the Care and Use of Laboratory Animals, and Subcommittee on Research Animal Care, Massachusetts General Hospital. Tap water and standard rat chow were provided ad libitum. Rats were randomly divided into burn and sham burn groups (n = 8 each). For the burn group, a 25% total burn surface area (BSA) full thickness burn injury was induced according to the procedures described previously.[2,9] Briefly, the animals were anesthetized with intraperitoneal injection of a mixture of ketamine (60 mg/kg) and xylazine (1.3 mg/kg). After shaving, a mark was drawn on the dorsal area according to a computer-generated template with the area equivalent to 25% of the calculated total body surface area.[9] The dorsal area

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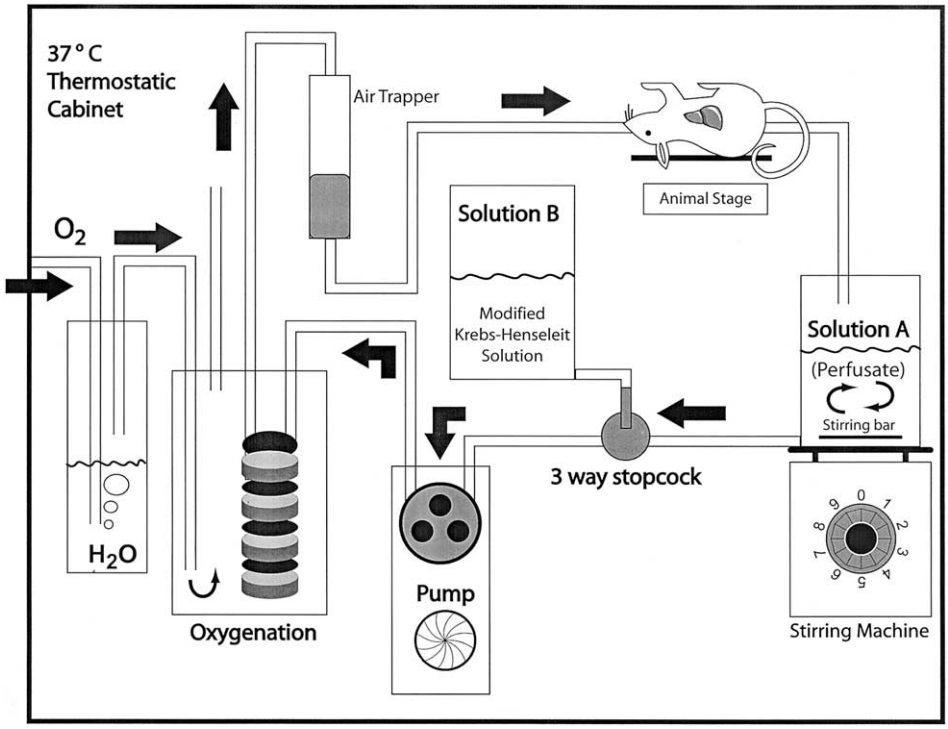

Fig 1. Liver perfusion system.

was then immersed in boiling water for 10 seconds along the predrawn mark. The full-thickness burn wound thus created has been verified by histology examination. This was followed by immediate fluid resuscitation by intraperitoneal injection of saline 3.0 mL · kg body weight^{-1} · %BSA^{-1}. The animals were under careful observation during their recovery from anesthesia. The sham burn animals were treated in the same manner except that the dorsal sites were submerged in a 37°C water bath for 10 seconds.

Perfusate and Isotope Preparations

Two perfusion solutions were prepared. Solution A, amino acid perfusate, was a modification of Eagle's Minimal Essential Medium conventionally used for hepatocyte culture (M0268; Sigma Chemical, St Louis, MO). The medium was mixed with dialyzed (Hemoflow F8; Fresenius USA, Lexington, MA) inactivated bovine serum albumin (Fraction V, Sigma Chemical Co) and a mixture of amino acid powders, to reach a final albumin concentration of 3%, and an amino acid profile (including glutamate, serine, alanine, proline, asparagine, aspirate, and glycine) and concentrations comparable to 2 times those in the portal vein.[2,10,11] The solution was then filtered through a 0.20 μm NALGENE disposable filter unit (Nalgene, Rochester, NY) for sterilization and preserved in sterile bags (300 mL of each) at −20°C until use. On the day of perfusion study, a stable isotope-labeled tracer L-[$^{15}N_2$-guanidino, 5,5-$^{2}H_2$]arginine .HCl (MassTrace, Woburn, MA) was added to the perfusate to reach a targeted enrichment of about 17.5% molar ratio. Solution B was a Krebs-Henseleit bicarbonate solution[12] containing 3% dialyzed (Hemoflow F8; Fresenius USA) inactivated bovine serum albumin (Fraction V, Sigma Chemical). It was also sterilized and stored at −20°C before use.

The liver perfusion system (Fig 1) was similar, albeit slightly modified, to one used previously.[2,13] The entire system was housed in a closed thermodynamic chamber at 37°C. Two separate containers, filled with either Solutions A (146.5 mL) or B (400 mL), were included. Before beginning the perfusion, each solution was circulated through this system for oxygenation and the pH adjusted to 7.4. The flow rate was determined based on 3.0 mL min^{-1} · g^{-1} of wet weight of the liver. The wet weight of the liver was estimated using the equation: $LV_{wet} = 0.034$ (body weight, kg)$^{0.89}$ before the infusion.[14] The exact flow rate during the perfusion was estimated by timed collecting and weighing the output.

The perfusion studies were performed on postburn (or sham burn) day 4. Cannulation of the portal vein was performed according to the procedures described previously.[2] Briefly, after laparatomy under ketamine and xylazine anesthesia, a #16 INTRACATH (Deseret, Sandy, UT) was inserted into the portal vein and secured with 3 "0" silk ties. Perfusion Solution B was immediately directed into the indwelled portal vein catheter with a maximum ischemia period of less than 1 minute. An incision was then made in the inferior vena cava at a site below the branching point of the right renal vein, and a loose tie placed around the inferior vena cava above its junction with the renal vein. The perfusion with Solution B continued until all residual blood was washed out of the liver. Simultaneously, a #14 INTRACATH was placed into the superior vena cava, secured, and the preset loose tie on the inferior vena cava was tightened immediately to redirect the liver output via the catheters in the superior vena cava. After a 20-minute perfusion with Solution B, the perfusate was switched to Solution A through a 3-way valve, and the output from the superior vena cava was directed into the reservoir containing Solution A. The recycling perfusion procedure was started and continued for 120 minutes. The whole perfusion procedures were conducted in a closed thermal regulated chamber maintaining at a temperature of 37°C.

A perfusate sample (2 mL) was taken from the container of Solution A before the addition of L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine tracer for the determination of baseline arginine enrichment. After mixing with the tracer, additional perfusate samples were taken at time 0 (before starting the perfusion) and then 10, 20, 40, 60, 90, and 120 minutes after starting the perfusion. These samples were preserved in −20°C freezer until analysis. In addition, 2 pairs of the perfusate samples were

also taken separately from the inflow and outflow lines of the liver at 60 and 120 minutes for the determination of oxygen content using a blood gas analyzer (238pH; CIBA Corning Diagnostic, Cambridge, MA). The volume of each perfusate samples taken during the perfusion was recorded. At the end of the study, the remaining perfusate in the reservoir was collected. These data were used to determine substrate utilization and metabolite production by the liver during the perfusion experiment. The liver was harvested and dried at −80°C in an incubator for 48 hours to obtain the dry weight.

Sample Analysis

The concentration of glucose in the perfusate was measured on a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). The concentration of urea was analyzed using a Sigma Diagnostic Kit (Sigma Diagnostics #640-A, Sigma Chemical).

The isotopic enrichments of the labeled arginine in perfusate samples were determined using the gas chromatograph-mass spectrometry technique, as described previously.[4,5] The arginine concentration in the perfusate was simultaneously determined using an isotope dilution method. In brief, 20 μL L-[U-$^{13}N_6$, U-$^{15}N_4$] Arginine.HCl(U-$^{13}N_6$,98%+; U-$^{15}N_4$,96% to 98%, concentration: 20.39μmol/L) was added to 200 μL of each perfusate sample as internal standard. The amino acids were extracted using the ion exchange column and prepared as methyl ester trifluoroacetyl derivative comparable to the one reported by Nissim et al.[15] The samples were then analyzed using on-column injection with a HP 5980 series II gas chromatograph coupled to a HP 5988A mass spectrometer (Hewlett-Packard, Palo Alto, CA). Selective ion monitoring (SIM) of arginine was conducted on the $[M\text{-}20]^-$ ion, using negative chemical ionization with methane as the reagent gas. Arginine was measured by SIM m/z 456, m/z 458, m/z 460, and m/z 466 for natural (M + 0), L-[5,5, $^2H_2$] arginine (M + 2), L-[$^{15}N_2$-guanidino, 5,5-$^2H_2$] arginine (M + 4) and L- [U-$^{13}N_6$, U-$^{15}N_4$] arginine (M + 10). The enrichments were corrected by multiple linear regression analysis on the data from standard samples containing known amounts of the arginine isotopomers (natural, M + 2 and M + 4).[16] The concentration was measured also against another standard curve based on varying known amounts of natural arginine against the same amount of internal standard (M + 10) arginine, based on the same principle for quantifying plasma ketoisocaproate concentrations previously described.[17]

The isotope abundance of [$^{15}N_2$]urea was measured in the perfusate with a tetra-butyldimethylsilyl (t-BDMS) derivative, using the electron – impact mode. SIM was performed at m/z 231 [M-57] for natural urea and at m/z 233 for [$^{15}N_2$] urea.

Calculations and Statistical Analysis

Oxygen consumption of the perfused liver was calculated from the difference in oxygen content between the inflow and outflow and the perfusion rate, expressed as micromoles per gram dry liver per minute.

Oxygen content of perfusate (O_2) = P_{O_2} (mm Hg) × 0.003 (mL O_2 · dL^{-1} · mm Hg^{-1}) = mL O_2 dL^{-1} perfusate.[18]

Oxygen consumption (μmol · g dry liver^{-1} · min^{-1}) = (inflow O_2 – outflow O_2) × flow rate (dL · g dry liver^{-1}) ÷ 22.4 (mmol · mL^{-1}) × 10^3 (μmol · mmol^{-1}).

The estimated rates of glucose, [$^{15}N_2$]urea and urea production were based on linear regression slopes for the change of their individual contents in the perfusion medium at different time points of the study (corrected for volume loss due to the sampling of the perfusate).

The metabolism of the exogenous arginine transported into the liver was quantified by tracing the metabolic fate of the L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine tracer. First, the rate of arginine uptake or transport into the liver was calculated from the fractional disappearance rate of total L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine from its initial total content in the perfusate. In brief, the remaining total amount of L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine in the perfusion medium at each time point during the perfusion process is converted to a fraction of its original amount at the start of perfusion. Then, the fractional rate of L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine disappearance (Fd*) is estimated by the slope of linear regression analysis. It follows that the unidirectional disappearance rates, or the transport rates of L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine (T_{arg}*) and total arginine (T_{arg}) from the perfusion medium can be calculated as:

$$T_{arg}* = Fd* \times [C_{arg}*] \quad (1)$$

where [C_{arg}*] is the total L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine content in the perfusion medium prior to starting the perfusion.

$$T_{arg} = Fd* \times [C_{arg}] \quad (2)$$

where [C_{arg}] is the total arginine content in the perfusion medium prior to starting the perfusion.

The fraction of the transported arginine utilized for urea production ($F_{A\text{-}U}$) is calculated by dividing the [$^{15}N_2$]urea appearance rate $Ra,_{urea}$* by T_{arg}* thus,

$$F_{A\text{-}U} = Ra,_{urea}/T_{arg}* \quad (3)$$

where $Ra,_{urea}$ is the slope of the linear regression of the total $^{15}N_2$ urea content in the perfusion medium at different time points.

The rate of urea production from the arginine transported from the extrahepatic source, U_{exh}, is then calculated by:

$$U_{exh} = T_{arg} \times F_{A\text{-}U}. \quad (4)$$

The rate of urea production from the intrahepatic source of arginine U_{inh} is calculated from the difference between the total rate of urea production ($Urea_{tot}$) and U_{exh} :

$$U_{inh} = Urea_{tot} - U_{exh} \quad (5)$$

Finally, the rate of the transported arginine entering the nonurea production pathway within the liver ($Arg_{non\text{-}urea}$) is calculated by:

$$Arg_{non\text{-}urea} = T_{arg} - U_{exh} \quad (6)$$

Data are expressed as mean ± SE. Student's *t* tests were used to compare the metabolism between 2 groups wherever indicated. Values of $P < .05$ were considered significant.

RESULTS

Rate of Oxygen Consumption and Glucose Production From the Liver

The liver dry weight from sham burn rats was 2.306 ± 0.072 g and that from burn rats was 2.202 ± 0.130 g ($P > .05$). The measured oxygen consumption rates at 60 minutes and 120 minutes of the perfusion period are given in Table 1. Oxygen consumption remained relatively constant during the perfusion period, and the rates did not differ between the 2 groups.

Table 1. Measured Oxygen Consumption During the Perfusion Study

Time Point of Measurement (min)	Rate of Oxygen Consumption	
	Sham Burn	Burn
60	3.1 ± 0.4	3.6 ± 0.4
120	3.8 ± 0.4	3.6 ± 0.3

NOTE. Data are expressed as mean ± SE, n = 8 for each group, in μmol · (g dry liver weight)$^{-1}$ · min^{-1}. No statistical difference with 2-way ANOVA test.

During the perfusion period, the concentration of glucose in the perfusion media showed a steady linear increase (Fig 2) as previously observed by Yamaguchi et al.[2] The glucose production rate in sham burn and burn groups did not differ significantly (30.7 ± 4.9 and 38.3 ± 4.0 μmol · g dry liver^{-1} · h^{-1}, respectively), although there was a tendency for the rate of the burn group to be higher.

Arginine Transport in the Perfused Liver

The initial concentration of arginine in the perfusate was 701.2 ±12.7 μmol/L in the sham burn group and 708.1 ± 14.0 μmol/L in the burn group ($P > .1$ by paired Student t test). These are comparable to the targeted concentration of 723 μmol/L. The enrichment of L-[$^{15}N_2$ guanidino, 5,5, $^2H_2$]arginine (molar ratio in excess of natural abundance) remained constant from beginning (mean ± SEM; 0.180 ± 0.006μ to end (0.180 ± 0.023) in the sham burn group; however, the enrichment in the burn group showed a slight, but significant, decline ($P < .05$) from 0.171 ± 0.007 at the beginning to 0.150 ± 0.009 by the end of 120 minutes of perfusion. This implies a slight release of intrahepatic arginine from the intrahepatic to the extrahepatic compartment after burn injury. During the recycling perfusion, there was a steady linear decline in the content of L-[$^{15}N_2$ guanidino, 5,5, $^2H_2$]arginine in the perfusate when expressed as a fraction of its initial content (Fig 3). The total amount of arginine in the perfusion medium showed a similar pattern of linear decline (data not shown). The rates of arginine transport from the perfusion medium to the liver and of urea production were calculated from the slope of these linear changes for both groups of animals and the initial arginine concentration based on Materials and Methods (equation 2). The results are summarized in Table 2. It was estimated that total arginine transport into the liver of burn rats proceeded at a rate about 3 times that for the sham burn group (Table 2).

At the end of the perfusion period, the enrichment of [5,5, $^2H_2$]arginine (M + 2 arginine) was below a reliable level of detection in the perfusates for both the sham burn and burn groups. This indicates that there was essentially no return into the extrahepatic space of the tracer arginine that had initially entered the urea cycle with conversion to urea and ornithine, with the latter reappearing as [5,5, $^2H_2$]arginine.

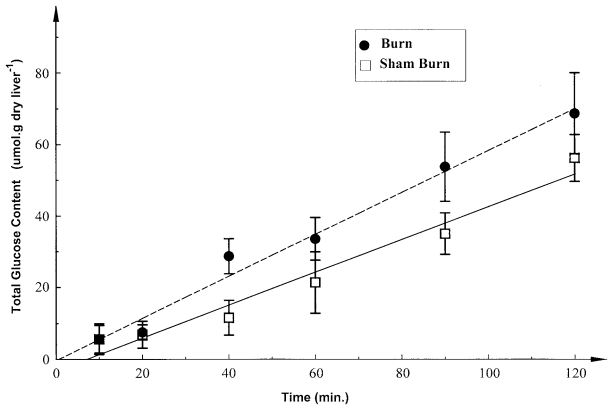

Fig 2. Glucose concentration and metabolism in the perfused liver.

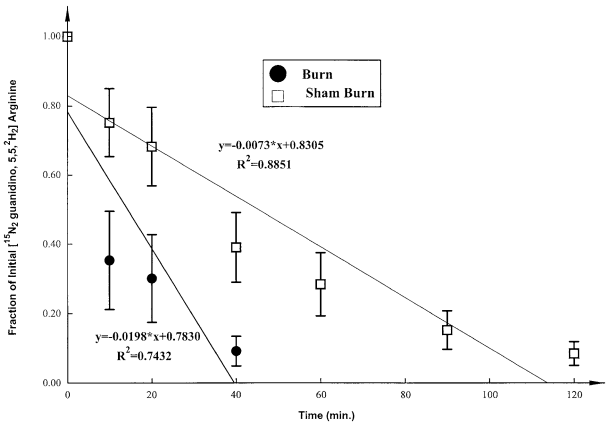

Fig 3. The fractional decline of total L-[$^{15}N_2$-guanidino, 5,5-$^2H_2$]arginine (averaged to per gram dry liver) in the perfusion media of the burned and sham burn groups.

Hepatic Urea Production and Its Relationship With Arginine Metabolism

The urea production rates based on linear regression analysis of total urea content in the perfusion media at different time points in the burn and sham burn groups (Fig 4) are also shown in Table 2. Although livers from both groups are perfused with a similar medium, the rate of total urea production was significantly higher in the burn group as compared with sham burn group ($P < .01$). Furthermore, the rate of net [$^{15}N_2$]urea production was also higher for the burned animals (Table 2), implying a higher rate of extrahepatic arginine being utilized for hepatic urea production. From the production rates of [$^{15}N_2$]urea and the transport rates of L-[$^{15}N_2$-guanidino, 5,5, $^2H_2$]arginine in these 2 groups, the fractions of transported arginine directed to urea production were found to be 46.3% ± 7.2% and 31.1% ± 5.2% ($P = .05$ by unpaired t test) in burn and sham burn groups, respectively. This implies a relatively higher fraction of liver arginine uptake being converted into urea after burn injury.

The relative contributions of extrahepatic versus intrahepatic arginine to total urea production in sham burn and burn rat livers were also quantified (Table 2). In sham burn animals, the extraheptic arginine accounts for a minor portion (<4%) of total hepatic urea production. Burn injury increased this proportion by approximately 3-fold, although still accounting for a relatively minor portion of total ureagenesis. The absolute values of urea production derived from extrahepatic arginine increased to 5-fold. This compares with a 25% increment in the utilization of intrahepatic arginine (Table 2).

Finally, the rate of the utilization of extrahepatic arginine in the liver via "nonurea production" pathways in these 2 groups of animals was also calculated (Table 2). The rate for the burned group was more than twice that of the sham group. The major intrahepatic "nonurea production" pathways for arginine utilization include formation of nitric oxide (NO),[19] agmatine,[20] creatine,[21] other guanido-containing substances,[22] and

Table 2. Arginine and Urea Metabolism in the Liver Measured During Perfusion

Metabolic Parameters	Sham Burn	Burn
Rate of arginine transport into liver	0.365 ± 0.021	1.269 ± 0.263†
Rate of [$^{15}N_2$ guanidino, 5,5, $^{2}H_2$]arginine transport	0.064 ± 0.004	0.208 ± 0.035†
Rate of [$^{15}N_2$]urea production	0.019 ± 0.002	0.093 ± 0.018*
Fraction of transported arginine used for urea production (%)	31.1 ± 5.2	46.3 ± 7.1‡
Rate of total urea production	3.235 ± 0.261	4.471 ± 0.274†
From extrahepatic arginine	0.109 ± 0.011	0.567 ± 0.124†
From intrahepatic arginine	3.127 ± 0.261	3.904 ± 0.295*
Fraction of Exogenous arginine to total urea production (%)	3.5 ± 0.4	12.9 ± 2.8†
Rate of the exogenous arginine entering nonurea synthesis pathway	0.257 ± 0.025	0.702 ± 0.185*

NOTE. Data are presented as mean ± SE; in μmol · (g dry weight)$^{-1}$ · min^{-1}, except where indicated.
*Significantly different from the sham burn group by unpaired t-test, $p < 0.05$ (unequal variance).
†Significantly different from the sham burn group by unpaired t-test, $p < 0.01$ (unequal variance).
‡$P = .05$, by unpaired *t* test, (equal variance).

its utilization for protein synthesis, of which protein synthesis is quantitatively the most important pathway.[14] Hence, it appears that the increased transport of exogenous arginine in the burn group may also be linked to the increased rate of protein synthesis in the liver, presumably including the synthesis of acute phase proteins. However, the proportion of arginine transported into the liver utilized by nonurea synthesis pathways showed a decrease in burned animals ($P = .05$).

DISCUSSION

The perfusion system used in this study is similar to that reported previously.[2] The perfusate is based on an oxygenated, hepatocyte culture medium. Pastor et al[23] used a hemoglobin-free perfusion system with graded level of oxygen content to determine the oxygen dependence of hepatic urea production. They established a "critical value of oxygen delivery rate" (D_{O2}crit) of 147 μL · min^{-1} · dry g^{-1}; below this the rate of ureagenesis decreased. The oxygen partial pressure in our perfusate was (mean ± SEM) 370 ± 30 mm Hg in the sham burn group and 346 ± 23 mm Hg in the burn group. These values remained unchanged at 10, 60, and 120 minutes (1-way analysis of variance [ANOVA], $P = .535$, and .215 distinctively for sham burn and burn groups). It can be calculated[18] that the oxygen content was about 11 μL mL^{-1}, and with a perfusion rate above 15 mL g dry weight^{-1} min^{-1}, the oxygen delivery rate exceeded the D_{O2}crit value established by Pastor et al.[23] In addition, the rate of glucose and urea production showed a linear increase during the perfusion experiment with the oxygen consumption remaining constant. It can further be estimated that the rate of urea production from the perfused liver in sham burn animals amounted to the equivalent of a total loss of urea nitrogen 1.2 gN · kg^{-1}. D^{-1}. This value is well within the range of reported values that can be derived from in vivo urinary nitrogen (mainly urea) excretion, eg,[24,25] as well as those for hepatocyte culture system.[26-28] This further supports the feasibility of our perfusion system for the present investigation.

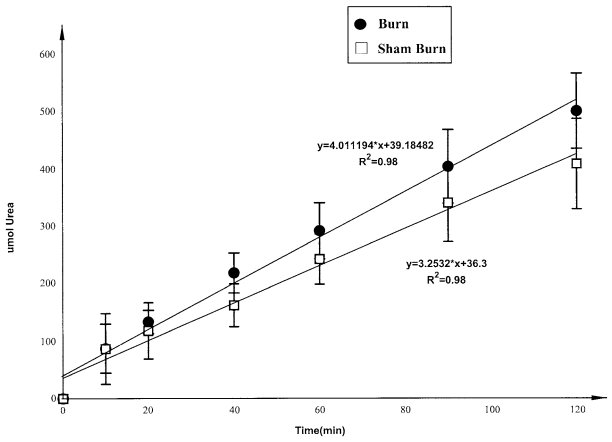

Fig 4. The rate of total urea appearance in the perfusion medium.

A concern in using a hemoglobin-free perfusion medium is the possible sharp decrease in oxygen delivery at the beginning of perfusion when the in vivo perfusion of the liver by whole blood is changed to the ex vivo perfusion with a hemoglobin-free medium. This might cause an immediate loss in the stability of intrahepatic glycogen, because a loss of hepatic glycogen content due to the use of a hemoglobin-free perfusate has been reported by Starnes et al.[29] However, because (1) the present study was aimed at exploring burn-induced metabolic changes with an emphasis on arginine and urea metabolism and (2), both burned and sham burned animals were studied after an overnight fast and treated with the same perfusion medium, the matters of glycogen stability would not appear to have an important bearing on our findings. Furthermore, recent investigations on hepatic glycogenolysis and other aspects of carbohydrate metabolism also have used similar hemoglobin-free perfusion media.[30-32]

The liver plays a central role in the regulation of the metabolic response to severe injury and trauma, with accelerated rates of gluconeogenesis and urea production being among the major metabolic aberrations seen after burn injury. The present study extends our previous investigation,[2] the overall purpose of which is to explore the factors that account for the altered hepatic nitrogen and energy metabolism after burn injury, with a focus on arginine and urea cycle activities. As in our previous study, perfused livers from burned rats demonstrated a similar rate of glucose output, but higher levels of urea production than

those from sham burn animals when they are perfused with similar media. It seems, therefore, that there is an intrinsic upregulation of the urea cycle activity and that this is not necessarily linked to the status of glucose metabolism. In the present study, we used stable isotope labeled L-[$^{15}N_2$-guanidino, 5,5, $^{2}H_2$]arginine tracer to explore in more detail the status of hepatic arginine metabolism in relation to the accelerated urea cycle activity. The arginine tracer allowed quantification of the unidirectional transport of arginine into the liver and of the rates at which this arginine entered different metabolic pathways. In addition, this paradigm yields information about the relative contributions of extrahepatic versus intrahepatic arginine to the accelerated rates of urea production and other relevant pathways after burn injury.

The present liver perfusion studies confirm that although arginine is the immediate precursor for urea synthesis, the extraheptic arginine pool contributes to a relatively minor fraction (less than 4%) of total urea production. This indicates a significant segregation of arginine metabolism among extrahepatic, intrahepatic nonurea cycle, and urea cycle compartments; and the channeling of arginine metabolism at the site of intrahepatic urea cycle activity. These findings are in line with the in vitro findings by Chueng et al,[3] which suggests that 3 extramitochondrial enzymes catalyzing the cytosolic portion of urea cycle activities are spatially grouped in close proximity to the mitochondrial membrane and organized in a way that the urea cycle intermediates are synthesized and metabolized in situ within the hepatocyte, with limited connections with arginine in the other compartments. Our findings with perfused livers are also consistent with our in vivo stable isotope tracer studies in human subjects, eg,[33-35] and animals,[36] which have demonstrated that plasma arginine turnover accounts for only a small fraction of total rate of hepatic urea production. Finally, there are 2 distinct isoenzymes of arginase encoded by different genes: arginase I (liver type), which is highly expressed in the liver as a component of the urea cycle, and arginase II (kidney type) located in the mitochondria,[37] which may also be expressed in liver and in other tissues.[38] It would be of interest to determine the relative contributions that these specific isoforms make to the increased rate of conversion of extrahepatic arginine to urea after burn injury.

The measured rate of arginine transport into liver of sham burn animals was about 0.365 μmol · g dry weight^{-1} · min^{-1}. Assuming the dry weight of the liver is mostly accounted for by its protein content, this estimate may be compared with a value of 0.234 μmol · g protein^{-1} min^{-1} for the arginine transport rate by the sodium independent y^+-system in plasma membrane vesicle preparations as reported by Pacitti et al.[8] The difference between the estimates may be explained by the existence of another nonsaturable arginine transport component, most likely a diffusion or high Km system,[3,39] which also contributes to the transport of arginine in vivo.

The present study also revealed that burn injury stimulated total hepatic arginine transport by nearly 3-fold. Again, it appears that this response of arginine transport to burn injury is also in line with previous findings that hepatic arginine transport by a hepatocyte plasma membrane vesicle system increased up to 2-fold in response to sepsis.[40,41] In the latter case , there was an upregulation of the membrane-bound arginine transport system y^+ in animals suffering from sepsis,[40] stimulated by inflammation mediators[8] and in response to thermal injury.[41] Because the intracellular arginine concentration is relatively low, due to the high intracellular arginase activity, especially under surgical stress,[42] it appears that the transport of extrahepatic arginine into hepatocytes plays an important role in determining the intrahepatic availability of arginine.

The present study further demonstrates that severe burn injury induced an accelerated inward transport of arginine, and that this is coupled with an increased utilization for urea production and via other pathways, presumably, with increased liver protein synthesis. In addition, there are at least 2 other intrahepatic pathways of arginine metabolism: the synthesis of NO via various isoforms of nitric oxide synthase[19] and the formation of agmatine via mitochondrial arginine decarboxylase.[43] It seems likely that an increased intrahepatic arginine availability is causally related to the production of nitric oxide and polyamine in burn patients because these metabolic pathways are stimulated in severe injury and sepsis-induced stress conditions.[44,45] Studies by Inoue et al[46] suggest that arginine transporters and the NO synthase enzyme may share a structurally similar arginine binding site. Hence, the increased arginine transport in sepsis is perhaps causally related to the increase in NO production, which in burns accounts for a stimulation of compensatory mechanisms for hemodynamic regulation and tissue repair in this condition. Billiar et al[47] also suggested that increased arginine availability for NO production may exert a protective effect against sepsis-induced oxidative damage to the liver tissue. The relationship between increased intracellular arginine availability and its protective role in reducing reactive oxygen species has been further demonstrated recently.[48,49] A more recent report on a series of liver perfusion studies by Nissim et al[20] demonstrated the contribution of extrahepatic arginine to the formation of agmatine, which plays an important role in modulating urea cycle activity via stimulating the synthesis of N-acetyl-glutamate. The latter is an obligatory effector in the initial step of urea synthesis, involving the conversion of NH_4^+ and HCO_3^- to carbamoyl phosphate by the mitochondrial enzyme carbamoyl phosphate synthetase I.[3] It is conceivable that increased hepatic arginine transport after burn injury may contribute to accelerated ureagenesis not only by directly increasing substrate supply for intrahepatic arginase, but also indirectly by activating the agmatine – acetyl-glutamate pathway. Further studies are warranted to explore the potential importance of this pathway in upregulating nitrogen loss after burn injury.

In studies with severely burned patients, we have found that the whole body net rate of de novo arginine synthesis is not upregulated, whereas the rate of arginine disposal and catabolism via oxidation of ornithine[6,7] is significantly increased. Thus, it is clear that an exogenous source of arginine is required to support arginine homeostasis in these patients, and the metabolic basis for this is illustrated by our findings that the contribution of extrahepatic arginine to total ureagenesis increases by about 3-fold after burn injury (Table 2).

In conclusion, the present study provides detailed quantita-

tive information about the metabolic relationships between the extrahepatic and intrahepatic arginine pools in association with the acceleration of the urea cycle activity after burn injury. Increased extrahepatic arginine transport into the liver and subsequently, its accelerated utilization for urea production and high rates of hepatic protein synthesis after burn injury are consistent with the notion that an enriched supply of exogenous arginine would likely be beneficial for improving the nutritional status of catabolic patients.

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