Splanchnic sequestration of amino acids in aged rats: in vivo and ex vivo experiments using a model of isolated perfused liver


1Laboratory of Biological Nutrition, René Descartes Paris 5 University; 2Clinical Chemistry, Hotel-Dieu Hospital Assistance Publique des Hopitaux de Paris, Paris; and 3Biology Laboratory, Emile Roux Hospital Assistance Publique des Hopitaux de Paris, Limel-Brevannes, France

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Jourdan M, Cynober L, Moinard C, Blanc MC, Neveux N, De Bandt JP, Aussel C. Splanchnic sequestration of amino acids in aged rats: in vivo and ex vivo experiments using a model of isolated perfused liver. Am J Physiol Regul Integr Comp Physiol 294: R748–R755, 2008. First published December 5, 2007; doi:10.1152/ajpregu.00291.2007.—Splanchnic sequestration of amino acids (SSAA) is a process observed during aging that leads to decreased peripheral amino acid (AA) availability. The mechanisms underlying SSAA remain unknown. The aim of the present study was to determine whether a high-protein diet could increase nitrogen retention in aged rats by saturating SSAA and whether SSAA could be explained by dysregulation of hepatic nitrogen metabolism. Adult and aged male Sprague-Dawley rats were housed in individual metabolic cages and fed a normal-protein (17% protein) or high-protein diet (27%) for 2 wk. Nitrogen balance (NB) was calculated daily. On day 14, livers were isolated and perfused for 90 min to study AA and urea fluxes. NB was lower in aged rats fed a normal-protein diet than in adults, but a high-protein diet restored NB to adult levels. Isolated perfused livers from aged rats showed decreased urea production and arginine uptake, together with a release of alanine (vs. uptake in adult rats) and a hepatic accumulation of alanine. The in vivo data suggest that SSAA is a saturable process that responds to an increase in dietary protein content. The hepatic metabolism of AA in aged rats is greatly modified, and urea production decreases. This result refutes the hypothesis that SSAA is associated with an increase in AA disposal via urea production.

Sarcopenia is the universal, involuntary decline in lean body mass that occurs with aging (30). The resulting muscle weakness and function loss increase the incidence of falls in the elderly, which increases occurrence of fractures and a decline in quality of life (31). Understanding the process of sarcopenia could allow new therapeutic strategies designed to prevent the loss of muscle mass and function, thus preserving the quality of life of elderly people and decreasing morbidity and mortality. The context of aging populations in Western countries makes this issue a major public health concern.

The underlying mechanism of sarcopenia is clearly multifactorial, and altered hormone secretion (54) and undernutrition and lack of adequate physical activity (11, 21) have been recognized as contributing factors. However, the metabolic alterations involved in sarcopenia remain poorly understood and require clarification. Sarcopenia mainly results from a slow, but continuous, decrease in protein synthesis in the postprandial state (56) resulting from a resistance to anabolic stimuli (50) and a decrease in substrate availability.

This last process is attributed to the splanchnic sequestration of amino acids (SSAA). Splanchnic extraction of leucine (6) and phenylalanine (49) is higher in elderly subjects studied in the postprandial state than in adults. The intensity of the splanchnic sequestration of leucine has been negatively correlated with leucine plasma levels in these elderly subjects (6). Recently, we reported the same effect in aged rats (17). Thus it appears that SSAA may be responsible for a decrease in the peripheral availability of dietary amino acids (AA), particularly for muscle protein synthesis, and is therefore a factor contributing to sarcopenia.

Since very little is known about SSAA, the aim of the present study was to gain a fuller understanding of this process. SSAA is a metabolic process. It must therefore have a limit and, therefore, can, in principle, be saturated. It is known that quantitative manipulation of nitrogen supply can modulate nitrogen retention in the elderly (3). Our first working hypothesis was accordingly that SSAA could be saturated by administration of a sufficient amount of protein. To test this hypothesis, we studied young and old rats fed a normal-protein diet (17% protein) or a high-protein diet (27% protein) for 14 days, and we measured nitrogen balance (NB) daily.

In addition, the precise role played by the gut and liver in SSAA is unknown. Three hypotheses can be advanced to explain the age-related increase in AA utilization in the splanchnic area: 1) an increase in protein synthesis in the gut and/or the liver, 2) an increase in AA transamination, and 3) an increase in AA disposal, mainly via urea production. In the fed state, the liver is the main organ of AA catabolism and, thus, plays a major role in the control of peripheral AA availability (26). We therefore focused our attention on our third hypothesis. Thus our second working hypothesis was that the liver, via a deregulated increase in AA catabolism reflected by an increase in ureagenesis, is the main organ involved in SSAA. To test whether the liver per se is responsible for alterations in nitrogen metabolism in old rats, we used the ex vivo isolated perfused liver (IPL) model in adult and old rats. This ex vivo model allows simulation of in vivo conditions and standardization of experimental conditions. This model was particularly useful for our present purpose, inasmuch as it enabled us to study age-related alterations in hepatic nitrogen metabolism...
independently of potential age-related alterations in gut metabolism.

METHODS

Animals

Young adult (3-mo-old) and old (24-mo-old) male Sprague-Dawley rats (Charles River, Saint-Germain-sur-l’Arbresle, France) were housed in individual metabolic cages. They were kept in a controlled environment (at a constant temperature of 21°C under a 12:12-h light-dark cycle) with ad libitum access to water. Sprague-Dawley rats are commonly used in aging studies (20, 25, 36, 46).

Experimental Design

The experimental design is shown in Fig. 1. During a 2-wk acclimatization period, all the rats were given ad libitum access to a standard chow diet that was recommended by the breeder (Charles River) as a maintenance diet for adult and old healthy rats (diet A04; Dietex, Villemoisson-sur-Orge, France). This standard chow, which contains 17% fish and vegetable protein, 3% lipids, 59% carbohydrates, and 21% water, fiber, vitamins, and minerals, was taken as the normal-protein diet. Daily spontaneous intake was recorded.

During a 2-wk experimental period (day 1–day 14), the rats were fed the normal-protein diet (17% protein) or a high-protein diet (27% protein). The high-protein diet, which was made up using A04 chow enriched with casein (Dietex) up to 27% protein, contained 2.6% lipids, 51.9% carbohydrates, and 18.5% water, fiber, vitamins, and minerals. We set the high-protein diet at 27% protein, because this level had been shown to increase protein accretion, even in malnourished old rats (52), and seemed reasonable in consideration of the decline of kidney function with aging.

The rats were divided into four groups on the basis of age and dietary nitrogen content during the experimental period: 1) young adult rats fed a normal-protein diet (AN group, n = 7), young adult rats fed a high-protein diet (AH group, n = 7), 2) old rats fed a normal-protein diet (ON group, n = 6), and 3) old rats fed a high-protein diet (OH group, n = 6).

To ensure complete food consumption, food was limited to 90% of the rats’ spontaneous food intake measured during the acclimatization period (28). The rats were weighed and urine was collected daily. On day 14 of the experiment, the animals were fasted for 6 h (but with free access to water) before liver isolation and perfusion (see below). This 6-h fasting period ensured that all the rats were in the postabsorptive state and facilitated the surgical procedure.

This research protocol complied with our institution’s guidelines for animal care. Two of the authors, L. Cynober and C. Moinard, are authorized by the French Ministry of Agriculture to use this experimental model (authorization nos. 005226 and 75522, respectively).

Liver Perfusion

Rats were anesthetized under controlled isoflurane (Baxter, Marcy, France) inhalation (Minervé, Esternay, France). Although positive (16) and adverse (45) effects have been described, there is no evidence that isoflurane affects protein metabolism in rats, and it remains the best anesthetic option in this species. The livers were prepared according to Miller’s technique, as previously described (33). Briefly, after cannulation of the bile duct, 1 ml of saline containing 500 IU of heparin (Héparine Choay, 25,000 IU/5 ml; Sanofi, Gentilly, France) was injected into the inferior vena cava, and the portal vein was cannulated. The liver was immediately rinsed with warmed oxygenated perfusion solution and excised. The isolated liver was perfused at constant pressure through the portal vein using a recirculating system in a thermostatically controlled cabinet (37 ± 0.5°C), where the perfusate was allowed to flow freely back into the tank via the hepatic veins. Portal pressure was maintained at the physiological level of 13 cm H2O by overflow of the perfusate into the tank.

The perfusate was a Krebs-Henseleit buffer supplemented with bovine serum albumin (30 g/l; VWR, Fontenay-sous-Bois, France), glucose (8.5 mM), and calcium (2 mM; Sigma, La Verpilliére, France) and oxygenated with 95% O2-5% CO2 (vol/vol). A solution of AA with antiproteolytic properties (alanine, glutamine, histidine, leucine, methionine, phenylalanine, proline, and tryptophan) was also added to the perfusate to control hepatic proteolysis (9). To ensure reliable liver AA exchange in near steady-state conditions, we compensated for hepatic AA extraction from 30 min (t0) by a bolus plus continuous AA infusion (0.2 ml/min), which allowed approximately twice the physiological AA levels to be maintained (10). At t0, 0.125 mg of indocyanine green (ICG, 2.5 mg/ml; SERB, Paris, France) was also added to the perfusate for measurement of ICG extraction.

Samples of perfusate were collected at 0, 35, 40, 45, 50, 60, 70, 80, and 90 min of perfusion for the measurement of biochemical parameters. The samples were stored at −80°C until direct analysis or deproteinization with sulfosalicylic acid (30 g/l) for AA analysis. Bile was collected at 30-min periods, and bile flow (μl/min) was estimated gravimetrically with the assumption of a specific mass of 1 g/ml. Portal flow (μl/min) and pH (Radiometer, Copenhagen, Denmark) of the perfusate (7.39 ± 0.05) were closely monitored throughout the experiment.

At the end of the perfusion phase, a small lobe of the liver was rapidly frozen in liquid nitrogen for measurement of intrahepatic AA. Total liver weight was then determined.

Analytical Methods

Urinary parameters. Urine samples were collected daily on a preservative (Amukin, Gifrer Barbezat, Decines, France). Urinary nitrogen output was quantified by chemiluminescence (Antek 7000, Alytek, Courtaboeuf, France) (13).

Urea was measured spectrophotometrically by a routine urease method (AU600 analyzer, Olympus, Rungis, France) (5).

Analyses performed on perfusate samples. BIOCHEMICAL PARAMETERS. Alanine aminotransferase activity was measured using the AU600 analyzer according to the recommendations of the French Society for Clinical Biology (5).

Urea was measured as described above.

AMINO ACIDS. AA in the perfusate were analyzed by ion-exchange chromatography with ninhydrin detection on an AA analyzer (Jéol, Croissy-sur-Seine, France) (32).

INDOCYANINE GREEN. ICG levels in the perfusate were measured using Nielsen’s spectrophotometric method (34), with correction for blank density at 900 nm.

AMINO ACIDS IN THE LIVER. To determine free intrahepatic AA concentrations, the samples were ground and deproteinized with 10%
trichloroacetic acid containing 0.5 mM EDTA (22). Supernatants were stored at −80°C until AA analysis (see above).

Calculations

Nitrogen loss in urine was measured daily from day 0 to day 14, and NB was calculated as the difference between nitrogen intake and urinary nitrogen output. Results are presented as daily and cumulative NB.

Relative nitrogen loss (RNL, %) was calculated as the amount of nitrogen excreted (Ne) per nitrogen ingested (Ni) at days 0 and 14:

\[ \text{RNL} = \left( \frac{N_e}{N_i} \right) \times 100 \]

The ratio of urinary urea nitrogen to total nitrogen (UUN/TUN) is presented as the average value calculated from day 1 to day 14:

\[ \text{UUN/TUN} = \left( \frac{\text{urinary urea}}{\text{total urinary nitrogen}} \right) \times 100 \]

where 2.8 represents the amount of nitrogen contained in one molecule of urea.

Hepatic exchanges of substrates were calculated for urea flux as:

\[ F = \frac{(L_2 \times V_2) - (L_1 \times V_1)}{t_2 - t_1} / E \]

and for AA flux as:

\[ F = \left[ \frac{(L_2 \times V_2) - (L_1 \times V_1)}{t_2 - t_1} - L_t \right] / E \]

where L_1 and L_2 are metabolite levels in the perfusion buffer at times t_1 and t_2, respectively, V_1 and V_2 are the volumes of perfusion buffer at t_1 and t_2 (to take into account volume variations due to sampling, evaporation, and addition), L_t is the infusion rate for a given amino acid, and E is the ICG extraction ratio. In a recent study (18), we demonstrated that this was a better mode of data expression than a ratio to liver weight; the ICG extraction ratio gives a better approximation of hepatic functional mass, total liver weight in old rats being higher than in young adults, irrespective of functional mass. Thus correction for this factor into takes into account age-related changes in hepatic extraction function.

The data are presented as the means of the fluxes calculated over three perfusion periods (t_15–t_45, t_45–t_90, and t_90–t_180).

Negative values represent metabolite uptake, while positive values represent metabolite release.

**ICG extraction ratio.** ICG removal from the perfusate was fitted to a biexponential curve \([C(t) = Ae^{-\alpha t} + B - \beta t}\] by nonlinear regression using Prism software (GraphPad, San Diego, CA). The macroconstants A, α, B, and β were thus determined to calculate ICG clearance (Cl) and ICG extraction ratio (E), as described by Vaubourdolle et al. (48):

\[ \text{Cl} = \frac{q_0}{A/\alpha + B/\beta} \]

\[ E = \text{Cl/Q} \]

where q_0 is the dose of ICG and Q is the perfusion flow.

**Statistics**

Values are means ± SE.

For NB measured at day 0 (i.e., before introduction of the high-protein diet in the AH and OH groups), Student’s t-test was used to compare adult and old groups.

For cumulative NB measured from day 1 to day 14, a two-way [age (A) and nutrition (N) or their interaction (A × N)] ANOVA for repeated measurements was performed to compare groups. Means were compared using the Newman-Keuls test.

For other parameters measured in vivo and parameters measured during IPL, a two-way ANOVA was performed to discriminate between aging effects, diet effects, and their interactions (A, N, and A × N). Means were compared using the Newman-Keuls test.

The StatView 5.0 software package was used. The level of significance was set at P < 0.05.

**RESULTS**

**Food Intake and Body Weight**

Food intake assessed during the acclimatization period was similar for all four groups: 24.34 ± 0.04, 23.82 ± 0.05, 23.87 ± 0.46, and 25.39 ± 0.36 g/day for AN, AH, ON, and OH groups, respectively.

Whole body weight was higher in old than in adult rats at day 14, whatever the diet given to the rats during the experimental period (400 ± 3, 399 ± 4, 665 ± 37, and 623 ± 39 g for AN, AH, ON, and OH groups, respectively, P < 0.05, old vs. adult rats fed the same diet), but not different between rats of the same age. The adult rats gained weight throughout the experiment, whereas total body weight remained stable in the old rats whatever the diet: 11.5 ± 0.7, 11.8 ± 0.8, −2.1 ± 0.4, and −1.2 ± 0.8 g for AN, AH, ON, and OH, respectively (P < 0.05, old vs. adult rats fed the same diet).

**NB, RNL, and UUN/TUN**

On day 0, all the rats were fed a normal-protein diet. In these conditions, NB was lower in old than in adult rats. This difference between adult and old rats fed a normal-protein diet was maintained throughout the experimental period (until day 14) expressed daily or as cumulative values (Fig. 2).

From day 1, the high-protein diet resulted in an increase in the NB of adult and old rats and abolished the age-related initial difference in NB observed at day 0. In adult rats, the increase in NB was transient (from day 1 to day 9), whereas in old rats the benefit of the high-protein diet was sustained throughout the experimental period (Fig. 2).

On day 0, RNL was significantly higher in old than in adult rats: 47.4 ± 2.5 vs. 70.2 ± 6.2% (P < 0.05). On day 14, this difference was maintained in the old rats fed a normal-protein diet but was abolished in the rats fed a high-protein diet: 42.9 ± 1.8, 60.4 ± 5.3, 65.3 ± 3.0, and 69.0 ± 4.5% for AN, AH, ON, and OH groups, respectively (P < 0.05, ON vs. AN and AH vs. AN).

UUN/TUN was similar for the four groups of rats: 85 ± 4.2, 89 ± 3.2, 85 ± 5.6, and 91 ± 5.7% for AN, AH, ON, and OH, respectively.

**Liver Characteristics and Viability**

Liver weight was higher in old than in adult rats (Table 1). Bile flow did not differ between groups and remained constant throughout the experiment. Portal flow was similar in all the groups. There was no diet effect on these variables.

Liver viability as assessed by alanine aminotransferase activity was well preserved in all the groups and was not affected by age or intake of the high-protein diet.

**Hepatic Metabolism**

**Urea production.** Urea production was significantly lower in old than in adult rats: 1.19 ± 0.18, 1.47 ± 0.34, 0.34 ± 0.03, 10.2 ± 0.32, 24.7 on June 28, 2017 http://ajpregu.physiology.org/ Downloaded from
Hepatic alanine content was four times higher ($P < 0.05$) in old than in adult rats (Fig. 3B). There was no diet effect on this hepatic accumulation of alanine.

**DISCUSSION**

The SSAA observed in elderly humans (6, 49) and in aged rats (17) may play a role in blunting postprandial stimulation of protein synthesis and, therefore, contribute to sarcopenia via a decrease in the peripheral availability of dietary AA. The present study was undertaken 1) to evaluate the response of SSAA to a high-protein diet by assessing nitrogen retention and 2) to clarify the role played by the liver in SSAA by describing age-related changes in hepatic nitrogen metabolism.

In the present study, the control group consisted of young adult (3-mo-old) rats. The choice of the age of animals for an adult reference group is still a subject of debate, because 1) the extrapolation of age of rats to humans is problematic, as elegantly discussed by Quinn (40), and 2) in contrast to humans, rats grow continuously. Nevertheless, most authors use 3-mo-old (1, 8, 27, 44) or 6-mo-old (38, 42, 43) rats as adult control groups, without any major difference in protein metabolism between animals at these two ages (35).

In agreement with a previous study in rats (52), we observed that when the rats were fed a normal-protein diet, aging was associated with a decreased NB corresponding to a lower nitrogen retention. This result does not reflect a net catabolic state in old rats, since their weight remained stable throughout the experiment. The excreted nitrogen remained mainly as urea (85–90%), whatever the age of the animal, ruling out effects of acidosis possibly linked to a decrease in renal function related to age or high protein intake. Although NB was lower in old rats, the percentage of nitrogen excreted per nitrogen ingested was higher in old than in adult rats. Thus AA oxidation was evidently increased in old rats. Taken together, these data obtained in the in vivo part of the present experiment thus support the concept of SSAA.

In the present study, NB was calculated by measurement of only urine nitrogen output. It is well known that nitrogen absorption is not a limiting factor over a wide range of protein intake (24), except when gut function is severely compromised (e.g., short bowel syndrome). Other losses (e.g., hair) are marginal.

In adult rats, the administration of a high-protein diet led to an increase in NB. However, this increase remained transient (from day 1 to day 9) and was followed by a normalization of NB corresponding to a metabolic adaptation leading to an increase in nitrogen loss. In humans, studies (39, 41) have shown that an increase in protein intake leads to increased nitrogen losses via higher AA oxidation, especially in the fed state. However, at day 14, although the percentage of nitrogen excreted per nitrogen ingested was higher in adult rats fed a high-protein diet than in those fed a normal-protein diet, it remained unchanged in old rats. These data suggest that nitrogen elimination capacity is already at its maximum in the old animals fed a normal amount of protein and that, in contrast to adult rats, they do not adapt their nitrogen losses to nitrogen intake.

Thus the extra nitrogen supplied by the high-protein diet was retained by the old rats, and SSAA clearly appears to be a saturable process. This conclusion is consistent with previous
LIVER ROLE IN SPLANCHNIC SEQUESTRATION OF AMINO ACIDS

Table 1. Influence of age and diet on characteristics and viability of the isolated liver

<table>
<thead>
<tr>
<th></th>
<th>Normal-Protein Diet</th>
<th>High-Protein Diet</th>
<th>Normal-Protein Diet</th>
<th>High-Protein Diet</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver wt, g</td>
<td>11.5 ± 0.4</td>
<td>12.1 ± 0.3</td>
<td>18.1 ± 1.0*</td>
<td>18.7 ± 1.3*</td>
<td>A</td>
</tr>
<tr>
<td>Bile flow, µl/min</td>
<td>4.2 ± 0.6</td>
<td>2.7 ± 0.3</td>
<td>3.4 ± 0.5</td>
<td>4.1 ± 0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Portal flow, ml·min⁻¹·g wet liver wt⁻¹</td>
<td>3.9 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.7 ± 0.1*</td>
<td>3.5 ± 0.1*</td>
<td>NS</td>
</tr>
<tr>
<td>ICG extraction ratio, %</td>
<td>3.6 ± 0.6</td>
<td>4.3 ± 0.5</td>
<td>8.1 ± 0.5*</td>
<td>11.2 ± 1.5*</td>
<td>A</td>
</tr>
<tr>
<td>ALT, IU/l</td>
<td>19.5 ± 1.8</td>
<td>23.5 ± 5.1</td>
<td>18.8 ± 4.5</td>
<td>17.8 ± 5.6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent wet liver weight at the end of perfusion and mean bile and portal flows throughout perfusion experiment. Livers from adult or old rats fed a controlled normal-protein or high-protein diet for 2 wk were isolated and perfused. ALT, activity of alanine amino transferase in the perfusate. *P < 0.05 vs. adult rats fed the same diet.

Table 2. Flux of selected amino acids

<table>
<thead>
<tr>
<th></th>
<th>Normal-Protein Diet</th>
<th>High-Protein Diet</th>
<th>Normal-Protein Diet</th>
<th>High-Protein Diet</th>
<th>Effect</th>
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<tbody>
<tr>
<td>Serine</td>
<td>66.9 ± 24.6</td>
<td>66.4 ± 20.0</td>
<td>22.5 ± 11.3</td>
<td>27.9 ± 4.7</td>
<td>A</td>
</tr>
<tr>
<td>Glutamate</td>
<td>239.9 ± 87.0</td>
<td>233.2 ± 54.9</td>
<td>134.9 ± 27.5</td>
<td>73.5 ± 11.7</td>
<td>A</td>
</tr>
<tr>
<td>Glutamine</td>
<td>-151.6 ± 92.3</td>
<td>-99.9 ± 84.9</td>
<td>2.6 ± 45.9</td>
<td>-58.2 ± 15.7</td>
<td>ns</td>
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<tr>
<td>Alanine</td>
<td>-363.2 ± 69.4</td>
<td>-308.1 ± 71.4</td>
<td>148.5 ± 59.8*</td>
<td>89.8 ± 32.5*</td>
<td>A</td>
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<tr>
<td>Proline</td>
<td>-136.9 ± 45.5</td>
<td>-73.4 ± 42.9</td>
<td>-17.2 ± 20.2*</td>
<td>-25.8 ± 11.7</td>
<td>A</td>
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<tr>
<td>Valine</td>
<td>25.6 ± 50.6</td>
<td>70.5 ± 30.9</td>
<td>31.3 ± 18.2</td>
<td>15.4 ± 9.1</td>
<td>ns</td>
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<tr>
<td>Isoleucine</td>
<td>16.4 ± 46.7</td>
<td>55.4 ± 28.2</td>
<td>27.5 ± 16.2</td>
<td>10.5 ± 9.2</td>
<td>ns</td>
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<tr>
<td>Leucine</td>
<td>28.9 ± 88.0</td>
<td>94.5 ± 52.7</td>
<td>40.6 ± 30.7</td>
<td>13.5 ± 15.6</td>
<td>ns</td>
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<tr>
<td>Arginine</td>
<td>89.0 ± 16.5</td>
<td>60.8 ± 15.1</td>
<td>-32.8 ± 7.5*</td>
<td>-30.3 ± 5.4</td>
<td>A</td>
</tr>
<tr>
<td>Citrulline</td>
<td>41.2 ± 10.8</td>
<td>36.4 ± 6.3</td>
<td>18.1 ± 1.9*</td>
<td>14.8 ± 2.8*</td>
<td>A</td>
</tr>
<tr>
<td>Ornithine</td>
<td>33.3 ± 7.5</td>
<td>26.3 ± 7.3</td>
<td>12.3 ± 1.8*</td>
<td>9.3 ± 2.0</td>
<td>A</td>
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</table>

Values (means ± SE) represent wet liver weight at the end of perfusion and mean bile and portal flows throughout perfusion experiment. Livers from adult or old rats fed a controlled normal-protein or high-protein diet for 2 wk were isolated and perfused. Amino acids were selected according to their relevance to the aim of the study. Negative values indicate uptake; positive values indicate release. Livers from adult or old rats fed controlled normal-protein or high-protein diet for 2 wk were isolated and perfused. Amino acids were selected according to their relevance to the aim of the study. Negative values indicate uptake; positive values indicate release. Livers from adult or old rats fed controlled normal-protein or high-protein diet for 2 wk were isolated and perfused. Effects were compared using Newman-Keuls test. *P < 0.05 vs. adult rats fed the same diet.

Observations in elderly humans (3, 49) and in aged rats (4). When an amount of AA higher than that normally provided by a meal was supplied orally to adult and elderly subjects, Volpi et al. (49) observed that muscle protein anabolism and arterial phenylalanine concentration were similar in the two groups, despite higher first-pass splanchnic extraction in elderly subjects. Katsanos et al. (19) demonstrated that aging was associated with diminished protein accretion after the ingestion of a small bolus of essential AA. Thus, in the study of Volpi et al. (49), the splanchnic sequestration of AA in elderly subjects may have been saturated. This would explain why, despite a higher first-pass extraction of phenylalanine, its arterial concentration was similar in elderly and young adults, and muscle protein synthesis was not affected. Also, in elderly women, the ingestion of 80% of daily protein intake as a single meal (the so-called pulse diet), which delivers a heavy protein load, improved NB more efficiently than spreading the same amount of protein across four isoproteic meals (spread diet) (3). The same protocol applied to old rats showed that the pulse diet led to higher plasma AA levels, together with a restored muscle protein synthesis response to feeding (4). Taken together, these results support the hypothesis that SSAA can be saturated above a certain threshold of protein or AA supply.

The mechanism underlying SSAA and the exact contribution of the liver and the gut in this process remain unknown. Since the liver is the main site of AA oxidation and urea production, we used an ex vivo study model, the IPL, to examine the hypothesis that age-related SSAA is caused by hepatic changes of nitrogen metabolism. One limit of our study is that we did not address the fate of AA in the intestine. This deserves further study via multicatheter techniques (14).

Urea synthesis is subject to three types of regulation: 1) flux of AA upstream of the cycle (9), 2) regulation by allosteric enzymes, and 3) hormonal regulation (26). We note that our model does not allow for differences in hormonal pattern (e.g., cortisol and proinflammatory cytokine) between adult and old rats. During IPL, the amount of nitrogen supplied to the liver was strictly controlled and kept equivalent across all four groups. Metabolic state was also the same across groups; i.e., all the rats were in the fasted state when the liver was isolated. These standardized experimental conditions enabled us to discriminate specific age-related metabolic alterations of the liver.

Metabolic (i.e., AA and urea) fluxes assessed during an IPL experiment are usually expressed per gram of wet liver. However, to allow the comparison of groups with different ages, correction by liver weight may not be sufficient, inasmuch as this may not reflect hepatic functional mass in old rats. Data from previous work (18) prompted us to reevaluate the mode of expression of metabolic fluxes. ICG extraction ratio, a surrogate of hepatic functional mass (53), was higher in old than in adult rats. Accordingly, to take into account age-related variations in liver functions (2), we normalized our data to ICG extraction ratio.
Remarkably, hepatic urea production was lower in old than in adult rats. This result refutes our second working hypothesis that SSAA is associated with an increase in AA disposal via urea production. An alteration of the fluxes of glutamine, arginine, or alanine (which are major precursors and/or inducers of urea) could potentially explain the altered ureagenesis in old rats. Aging had no effect on the hepatic flux of glutamine, which is a possible reason for the increase in nitrogen elimination observed in vivo in old rats. This result supports the idea that SSAA is associated with an increase in AA disposal via urea production. The liver per se does not appear to be the main organ involved in SSAA. Extrahepatic factors may be responsible for the increased nitrogen elimination observed in vivo in old rats. The intestine may hold the key, since this is where arginine and glutamine, the main inducers and substrates of ureagenesis, are metabolized to citrulline, which does not

Table 3. Levels of selected amino acids in the liver

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<tr>
<th></th>
<th>Normal-Protein Diet</th>
<th>High-Protein Diet</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serine</td>
<td>0.30±0.03</td>
<td>0.18±0.02</td>
<td>A×N</td>
</tr>
<tr>
<td>Glutamate</td>
<td>2.37±0.17</td>
<td>2.56±0.24</td>
<td>NS</td>
</tr>
<tr>
<td>Glutamine</td>
<td>3.90±0.41</td>
<td>3.27±0.35</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>2.22±0.29</td>
<td>1.86±0.21</td>
<td>A</td>
</tr>
<tr>
<td>Proline</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>0.38±0.01</td>
<td>0.46±0.03</td>
<td>N</td>
</tr>
<tr>
<td>Isoeucine</td>
<td>0.27±0.01</td>
<td>0.33±0.02</td>
<td>N</td>
</tr>
<tr>
<td>Leucine</td>
<td>0.67±0.01</td>
<td>0.76±0.05</td>
<td>A×N</td>
</tr>
<tr>
<td>Arginine</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Citrulline</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ornithine</td>
<td>0.30±0.02</td>
<td>0.23±0.02</td>
<td></td>
</tr>
</tbody>
</table>

Values (means ± SE) represent content of each amino acid in the liver (μmol/g liver). Livers from adult or old rats fed controlled normal-protein or high-protein diet for 2 wk were isolated and perfused. ND, not determinable. Amino acids were selected according to their relevance to the aim of the study. Other amino acids did not show relevant significant difference. A 2-way ANOVA with 2 factors (A and N) or their interaction (A×N) was performed to compare groups. Means were compared using Newman-Keuls test. *P < 0.05 vs. adult rats fed the same diet. †P < 0.05 vs. rats of the same age fed normal-protein diet.
undergo hepatic uptake (7). Intestinal citrulline production from arginine and glutamine has been shown to be dependent on protein intake (51). Taken together, these data suggest that interorgan AA exchanges between the gut and liver may be key factors in achieving a normal stimulation of ureagenesis during aging.

GRANTS

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REFERENCES


