Metabolism of ornithine, α-ketoglutarate and arginine in isolated perfused rat liver

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Ornithine (Orn; α-ketoglutarate (αKG) salt) and arginine (Arg) supplementation of enteral diets has been advocated in the treatment of hypercatabolism of trauma patients, but both compounds are subject to extensive hepatic metabolism. To compare the metabolism of these two compounds and to evaluate the possible influence of the αKG moiety, livers were perfused with αKG, Orn, ornithine α-ketoglutarate (OKG) or Arg (n 6 in each group) for 1 h. Arg uptake was nearly fourfold higher than Orn uptake (690 (SD 162) v. 178 (SD 30) nmol/min per g liver), and Orn uptake was not modified by αKG. Orn was totally metabolized by the liver, whereas Arg led to Orn release (408 (SD 159) mmol/min per g liver) and a threefold stimulation of urea production (Arg 1·44 (SD 0·22) v. Orn 0·45 (SD 0·09) μmol/min per g liver). αKG alone only increased hepatic aspartate uptake but, when associated with Orn as OKG, it led to an increase in glutamate release and in proline content in the liver and to a decrease in proline uptake. From these findings we conclude that (1) Arg load is extensively metabolized by the liver, inducing urea production, (2) in enteral use, Orn supplementation appears preferable to Arg as it is less ureogenic (as also recently demonstrated in vivo in stressed rats receiving isomolar amounts of Arg and Orn), (3) the liver participates in the Orn–αKG metabolic interaction, mostly in proline metabolism, which occurs in the splanchnic area.

Arginine: α-Ketoglutarate: Liver: Ornithine

Nutritional support has emerged as an important component of the treatment of severely ill patients, but there is a need for qualitative improvements through the use of compounds which can modulate protein turnover. In this respect, ornithine (Orn), as its α-ketoglutarate (αKG) salt, and arginine (Arg) are promising candidates and several studies have provided firm evidence that supplementation of enteral or parenteral nutrition with ornithine α-ketoglutarate (OKG; Cynober, 1991) or Arg (Barbul, 1986, 1990) improves nutritional status and immunity of hypercatabolic patients.

Arg and Orn could act on protein metabolism through either increased protein synthesis or decreased protein catabolism (Barbul, 1986, 1990; Cynober, 1991), but the precise mechanism involved is unclear. Concerning OKG, it has been suggested that αKG uptake by muscle leads to glutamine formation (Hammarqvist et al. 1990; Wernerman et al. 1990) which in turn inhibits proteolysis or stimulates protein synthesis (Rennie et al. 1986). Polyamine formation from Orn, also, could be involved (Lescoat et al. 1987; Vaubourdolle et al. 1990), since these substances play a key role in the control of protein synthesis and cell growth (Grillo, 1985). Finally, it has been shown that αKG modifies Orn metabolism towards proline and Arg formation (Cynober et al. 1990). However, since Orn and Arg are closely associated in the urea cycle, it is not known if Orn acts via Arg formation or vice versa.
If a direct peripheral effect of these molecules is involved, a factor of choice for nutritional support would be their ability to escape degradation in the splanchnic area, i.e. their availability for peripheral tissues such as muscle and cells of the immune system. Although Orn and Arg are both extensively metabolized in the splanchnic area after enteral, intravenous and intraperitoneal administration (Vaubourdolle et al. 1988, 1989; Blachier et al. 1991), there is, to the best of our knowledge, no direct comparison of their behaviour. The main aim of the present study was to investigate the hepatic metabolism of Orn and Arg. As it has been shown (Cynober et al. 1990) that aKG modifies Orn metabolism, we also tried to establish whether the liver is the site of the aKG-Orn interaction. The isolated perfused rat liver was chosen since it is an integral model, especially with regard to cooperation between perivenous and periportal cells (Haussinger, 1989). Experiments were done under physiological conditions appropriate for amino acid flux (De Bandt et al. 1990, 1991).

MATERIALS AND METHODS

Chemicals

OKG (Cetornan®) was kindly provided by Laboratoires Logeais (Issy-les-Moulineaux, France). Sodium α-ketoglutarate, ornithine hydrochloride and Arg (free base) were from Sigma (La Verpillière, France). Other amino acids used for liver perfusions were obtained from Sigma. Other reagents and products necessary for liver perfusion were obtained from various sources (De Bandt et al. 1990, 1991).

Liver perfusion

Livers from overnight-fasted male Sprague-Dawley rats (body weight 276 (SD 30) g, n 30) were prepared for the perfusion as previously described (De Bandt et al. 1991). Briefly, after cannulation of the bile duct, 1 ml saline (9 g NaCl/l) containing 1000 U heparin was injected into the saphenous vein and then the portal vein was cannulated. The liver was perfused immediately through the portal vein with 30 ml of the perfusion solution and excised. The liver was then perfused at constant physiological pressure for 90 min in a thermostatically controlled cabinet (37°C). The perfusate was Krebs-Ringer buffer supplemented with albumin (30 g/l), Ca (2 mmol/l) and glucose (7.5 mmol/l). The perfusion medium (150 ml) was recirculated and oxygenated with O₂-CO₂ (95:5, v/v); pH was maintained at 7.4. As previously described (De Bandt et al. 1991), a mixture of amino acids with antiproteolytic properties (Ala, Leu, Gln, Pro, Phe, His, Trp, Met at twice their physiological plasma concentrations) was added at the beginning of the perfusion. At the end of a 30 min equilibration period (tₐ₀) a balanced amino acid mixture supplemented with glutamine (0.85 mmol/l) but devoid of Arg was added in a quantity designed to be approximately twice the physiological concentration for all amino acids.

Depending on the experiment, the perfusate was supplemented at tₐ₀ with either Orn (4 mM), OKG (2 mM, OKG containing 2 mol Orn per 1 mol aKG), aKG (2 mM) or Arg (4 mM). Results were compared with those of a control group (n 6 for each group).

Samples and measurements

Samples of perfusate were taken at tₐ₀ (before addition of the balanced AA mixture and test compounds) and then at 35 (tₐ₅), 45 (tₐ₅), 60 (tₐ₆) and 90 (tₐ₉) min. They were stored at -20°C either immediately or after deproteinization with sulphanilic acid (50 mg/ml) for the determination of amino acids.
HEPATIC METABOLISM OF ORNITHINE AND ARGinine

At the end of the perfusion, portions of liver were quickly frozen in liquid N₂ and stored at -80° for the measurement of amino acid content.

Individual free amino acid concentrations were measured by ion-exchange chromatography on a System 6300 analyser (Beckman, USA).

Glucose, urea and NH₃ were measured by standard enzymic methods on a Progress analyser (Kone, Finland).

αKG was measured by the method of Burlina (1985).

For intrahepatic amino acid determinations, frozen tissue samples were homogenized in ice-cold (40 ml/l) HClO₄-0·25 mM-EDTA and amino acids were measured as described above.

Substrate exchanges were calculated as:

\[
\frac{(Ct_2 V_2 - Ct_1 V_1)}{(t_2 - t_1) \times W},
\]

where \( C \) and \( V \) are the substrate concentrations in two consecutive samples at time intervals \( t \), \( V_1 \) and \( V_2 \) are the volume of perfusate at the same time intervals and \( W \) is the liver wet weight. This formula takes into account changes in perfusate volume due to sampling and evaporation (0·2 ml/min). When fluxes were evaluated over longer periods (e.g. from \( t_8 \) to \( t_{40} \)) they were calculated as the means of successive determinations.

Statistical analysis

Results are presented as means and standard deviations.

The five groups were compared using the Kruskal–Wallis and Mann and Whitney tests on PCSM software (Deltasoft, Grenoble, France). \( P \) values of 0·05 or less were considered significant.

RESULTS

Ornithine α-ketoglutarate and arginine liver fluxes

The uptake of Orn was lower than that of Arg (Table 1, Fig. 1) and was not modified by αKG (Fig. 2). αKG was significantly taken up by the liver and this uptake was not modified by Orn (Table 1, Fig. 2). Fluxes calculated over the whole experimental period \( (t_{38} - t_{40}) \) indicated that, of the molecules studied, Arg was the molecule most extensively taken up by the liver (Table 1). Arg also led to strong Orn release (corresponding to 60% of the Arg taken up by the liver; Fig. 1).

Amino acid flux in the perfused liver

Arg induced an increase in aspartate and histidine uptake (Table 1). After Orn administration no Orn metabolites were detectable in the medium. However, Orn increased aspartate uptake to a similar extent to Arg and reduced tyrosine and glutamate release. Compared with Arg, Orn also reduced serine, glycine and methionine uptake.

αKG had limited effects on liver amino acid fluxes, only aspartate uptake increased. However, when combined with Orn as OKG, αKG also led to a reduction in proline uptake, and prevented the reduction in glutamate release induced by Orn. Although some specific amino acids fluxes were affected, total amino acid flux was not statistically different between the five groups.
End products of amino acid metabolism

Compared with control values, urea production was greatly increased by Arg (nearly 20-fold) and by Orn (6-fold), whereas αKG had no effect (Fig. 3). NH₃ production was decreased by both Orn (alone or as OKG) and Arg (Fig. 4). These effects were accompanied by a modification of the hepatic N balance (Table 2); the rate of N release: the rate of N uptake was approximately 50% in the control group; it was increased by Arg and to a lesser extent by Orn (alone or as OKG).

Only Orn, alone and as OKG, suppressed the glucose uptake observed in the control group (−36 (SD 47) and −14 (SD 35) nmol/min per g liver respectively v. 127
Table 1. Effects of α-ketoglutarate (αKG), ornithine α-ketoglutarate (OKG), ornithine (Orn) and arginine (Arg) on amino acid and αKG hepatic fluxes (nmol/min per g liver wet weight)† in rats‡.

(Values are means and standard deviations for average fluxes determined over the entire experimental period)

<table>
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<tr>
<th>Treatment group...</th>
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<tr>
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| a,aa,ca,d,dd Mean values were significantly different: v. control: a P < 0.05, aa P < 0.01; v. OKG: c P < 0.01; Arg v. Orn: d P < 0.05, dd P < 0.01. |
| ΣAA, global amino acid flux minus Orn and Arg. |

Differences between the five groups were significantly different (Kruskal-Wallis): * P < 0.05, ** P < 0.01, *** P < 0.001.

† Positive values represent uptake and negative values represent release.
‡ For details of experimental procedures, see pp. 228–229.
Fig. 3. Effects of ornithine (Om), α-ketoglutarate (aKG), ornithine α-ketoglutarate (OKG) and arginine (Arg) on hepatic ureagenesis in rats. (△—△), Control; (△—□), Om; (□—□), aKG; (□—△), OKG; (○—○), Arg. Points are means and standard deviations represented by vertical bars for six perfusates per group. Mean values were significantly different from those for control group: *P < 0.05, **P < 0.01. Mean values were significantly different from those for Om group: †P < 0.05, ††P < 0.01. For details of experimental procedures, see pp. 228–229.

Fig. 4. Effects of ornithine (Orn), α-ketoglutarate (aKG), ornithine α-ketoglutarate (OKG) and arginine (Arg) on hepatic ammonia disposal in rats. (△—△), Control; (△—○), Orn; (□—□), aKG; (□—△), OKG; (○—○), Arg. Points are means and standard deviations represented by vertical bars for six perfusates per group. Mean values were significantly different from those for control group: *P < 0.05, **P < 0.01. For details of experimental procedures, see pp. 228–229.

(SD 95) nmol/min per g liver in controls; P < 0.05). In the Arg-treated group, glucose flux was similar to the control value (values not shown).

**Intrahepatic amino acids**

Arg and Orn increased intrahepatic glutamine, citrulline and Orn content (Table 3).

In the presence of aKG, Orn led to a marked decrease in intrahepatic aspartate, serine (v. Orn and aKG), and in alanine, threonine, glutamate, lysine and histidine (v. aKG) and to an increase in proline content (Table 3).

Arg was never detectable in the liver.
Table 2. Effects of α-ketoglutarate (aKG), ornithine α-ketoglutarate (OKG), ornithine (Orn) and arginine (Arg) on hepatic nitrogen fluxes (nmol/min per g liver wet weight)* in rats†

(Values are means and standard deviations for average fluxes determined over the entire experimental period)

<table>
<thead>
<tr>
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<th>OKG</th>
<th>Orn</th>
<th>Arg</th>
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<td>Arg-N flux</td>
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<td>-882</td>
<td>-918</td>
<td>-2903</td>
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<tr>
<td>Extra N release‡</td>
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<td>-0.64</td>
<td>-0.70</td>
<td>-0.75</td>
<td>-0.96</td>
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<tr>
<td>N uptake§</td>
<td>0.05</td>
<td>0.12</td>
<td>0.03</td>
<td>0.06</td>
<td>0.05</td>
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</tbody>
</table>

* Positive values represent uptake, negative values represent release.
† For details of experimental procedures, see pp. 228–229.
‡ Calculated as urea plus HN₄-N release.
§ Calculated as N release minus N release in the control group.

Mean values were significantly different: v. control: a P < 0.05, aa, P < 0.01; v. Orn: c P < 0.01.

ΣAA-N, global amino acid-N flux minus Orn and ARG.
Table 3. Effects of α-ketoglutarate (αKG), ornithine α-ketoglutarate (OKG), ornithine (Orn) and arginine (Arg) loads on intrahepatic amino acid concentrations (nmol/g liver) in rats.
(Mean values and standard deviations for six perfusates per group)

<table>
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<th>OKG</th>
<th>Ornithine</th>
<th>Arginine</th>
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</table>

**Mean values were significantly different:** v. control: a P < 0.05, aa P < 0.01; OKG v. αKG: b P < 0.05, bb P < 0.01; Orn v. OKG: c P < 0.05.
ND, not detected (< 50 nmol/g).
* Differences between the five groups were significantly different (Kruskal-Wallis): * P < 0.05, ** P < 0.01.
+ For details of experimental procedures, see pp. 228-229.
Hepatic metabolism of ornithine and arginine

Discussion

To the best of our knowledge, the present study is the first to compare Arg and Orn uptake and metabolism in the isolated perfused liver. It provided new insight into the metabolism of these amino acids since the model used allowed amino acid fluxes similar to those observed in vivo to be obtained (De Bandt et al. 1990, 1991).

Ornithine, arginine and α-ketoglutarate

An unexpected finding of our study was that Arg was extensively taken up by the liver, while Orn uptake was comparatively low. These results contrast with the general assumption that cationic amino acid uptake by the liver is limited. This opinion is supported by the low activity of the $y^+$ transport system, shared by Arg and Orn, in the hepatocytes (Moseley, 1993). However, as demonstrated by White & Christensen (1982), system $y^+$ contributes little to the flux of cationic amino acids in the hepatocytes and a non-saturable transport component exists whose contribution seems important relative to the system $y^+$. This diffusional component of Orn transport has been demonstrated in Ehrlich-cell plasma-membrane vesicles (Medina et al. 1991) as well as in hepatocytes (Metoki & Hommes, 1984), and its importance increases with increasing Orn concentrations (Metoki & Hommes, 1984). Since our experiments were performed at high Orn and Arg concentrations (4 mM), the uptake of these two compounds could tentatively be attributed to this non-saturable transport. The higher uptake of Arg could be explained by differences in subsequent metabolism. Arginase (EC 3.5.3.1) levels are very high in the liver, maintaining a very-low free Arg concentration, and an Arg gradient between extra- and intracellular compartments would, thus, favour arginine uptake; conversely, Orn conversion to citrulline is a rate-limiting step leading to Orn accumulation.

While Arg uptake was higher than that of Orn, Arg was promptly released as Orn. This observation could explain the results of Saito et al. (1987) who gave a diet containing 0, 10, 20 or 40 g Arg/kg enterally to burned guinea-pigs. Plasma Orn increased as a function of Arg dose and the increase in Orn was even higher than the increase in plasma Arg itself. Daly et al. (1988) administered 25 g Arg/d enterally to surgical patients. The increase in plasma Orn was higher than that in Arg (from 57 to 259 μmol/l and from 87 to 213 μmol/l respectively). These authors concluded that the beneficial effects of Arg on the immune system might have resulted from Arg conversion into Orn. While the mechanism of action of Orn and Arg on immune status is unclear, it seems reasonable to assume that their effects are at least in part dependent on metabolism in target tissues such as muscle, immune cells and all rapidly dividing cells (the multiplication of which is dependent on the polyamines synthesized from these amino acids (Grillo, 1985)). From this point of view, lower liver uptake appears to be a favourable characteristic since it increases extrasplanchnic availability.

The uptake of αKG is in agreement with the findings of Stoll & Haussinger (1989) who showed rapid uptake by the liver (93 (SD 11) vs. 87 (SD 36) nmol/min per g in our study).

Ornithine and arginine metabolism

Both Orn and Arg are anti-ammoniogenic (Barbul, 1986; Molimard et al. 1968), due to efficient recycling of NH₄ into ureagenesis. This acceleration of ureagenesis could explain the increase in aspartate uptake, and the decrease in its intrahepatic concentration since this amino acid is the second N donor of urea cycle. Although Arg is required for hepatic N disposal (as shown by the suppression of NH₄ release in the Arg-treated group), it is also a strong activator of urea synthesis and leads to an increase in amino acid catabolism: (a)
in the control group, N release: N uptake averaged 50\% (Table 2), which indicates that approximately half the amino acid-N taken up remained in the liver; (b) in the Orn group, the catabolism of Orn could explain the increase in this ratio since the extra N supplied as Orn (357 nmol N/min per g liver) approximately matches the increase in N release (extra N release 434 nmol N/min per g liver; Table 2); (c) in the Arg group, the N produced by the complete catabolism of Arg (Arg-N uptake minus Orn-N release 1944 nmol N/min per g liver) could only account for 80\% of the extra N release (2419 nmol N/min per g liver; Table 2). The remainder (475 nmol N/min per g liver) must have arisen from increased catabolism of other amino acids.

It must be mentioned that in vivo the rate of urea synthesis would probably be higher. Indeed, it has been demonstrated in fed rats as well as in humans that portal vein NH$_3$, derived from a protein meal, contributes about 30\% to urea synthesis (Meijer et al. 1990). Thus, it would be interesting to determine whether an extra NH$_3$ load could affect Arg-induced amino acid catabolism.

Our results can be compared with those of Saheki et al. (1975) who studied the regulation of urea synthesis from NH$_4$Cl in the perfused livers of rats subjected to different dietary conditions. In the liver of rats fed on a low-protein diet, the rate of urea formation relative to the rate of NH$_3$ removal was 36.1\% (calculated as N equivalent), suggesting an anabolic utilization of N. This value was increased to 63.2\% by Orn and N-acetylglutamate and to 91\% in the liver of rats fed on a high-protein diet. Our findings further support the hypothesis that Arg could act as a regulatory signal to the liver relating to dietary protein intake (Morimoto et al. 1990).

However, the mechanism by which Arg modulates ureagenesis is still much debated: (a) Meijer et al. (1990) and Morimoto et al. (1990) have proposed that N-acetylglutamate acts as the main regulator of carbamoyl phosphate synthase I (EC 6.3.4.16; CPS); the acceleration of urea production in our experiments would then result from the activating effect of Arg on N-acetylglutamate synthetase (Meijer et al. 1990). However, the relationship between N-acetylglutamate content and CPS activity rate seems more complex than would be expected (Beliveau-Carey et al. 1993); (b) Lund & Wiggins (1986) have postulated that Orn availability is the principal regulator of ureagenesis. Orn could be implicated through increased conversion to glutamate and then to aspartate, or through increased intramitochondrial transport, leading to the activation of CPS. The higher uptake of Arg v. Orn, through an increase in intracellular Orn availability, thus would be responsible for the higher rate of urea synthesis in the Arg-treated group v. Orn-treated group. Alternatively, it has been suggested (Meijer et al. 1990) that arginase, CPS and ornithine carbamyltransferase (EC 2.1.3.3; OCT) are physically closely associated thus, exogenous Arg is a better precursor for citrulline synthesis than Orn and Orn is made available for the decarboxylation and transamination pathways (Cynober, 1994). However, the fact that although Arg increases liver Orn (33.3\%) and citrulline (35.8\%), liver citrulline: Orn content is similar (about 0.045) after Orn and Arg loads does not favour this hypothesis.

Thus our findings point to a further advantage of Orn over Arg: Orn appears markedly less ureogenic. In whole-body terms this finding must be evaluated according to the possible extrahepatic conversion of Orn to Arg. Interestingly, we recently observed (Le Boucher et al. 1992) that supplementation of the enteral diet of burned rats with high doses of Arg increased plasma urea much more than an equimolar dose of Orn. The extrahepatic conversion of Orn to Arg would first require transformation to citrulline through the action of OCT, and the two-step conversion to Arg through argininosuccinate synthase (EC 6.3.4.5) and argininosuccinate lyase (EC 4.3.2.1). However, in the kidney (the main organ of de novo Arg synthesis) OCT is only present in trace amounts (Windmueller &
Spaeth, 1981) and Arg release correlates with citrulline uptake by the kidney of rats infused with citrulline (Dhanakoti et al. 1990). Moreover, since in our experiments 60% of Arg taken up by the liver is converted to Om, this Om would have the same fate as exogenous Orn. An alternative pathway would be the intestinal transformation of Orn to citrulline; however, while enteral administration of Arg leads to the production of urea and citrulline (Windmueller & Spaeth, 1976; Rerat et al. 1988), labelled citrulline was not found in the intestine after enteral administration of [14C]Orn to rats (Vaubourdolle et al. 1989) and in fact [14C]citrulline was located exclusively in the liver.

The Arg-induced acceleration of amino acid metabolism and the urea cycle implicates an increase in bicarbonate utilization by the liver (Meijer et al. 1990). Thus, it would be preferable to give Orn rather than Arg to catabolic patients who exhibit a compromised N status and a tendency to acidosis. In addition, the use of Orn as its αKG salt will be of further interest in these situations since αKG enhances renal H⁺ secretion (Welbourne, 1993) and OKG administration counteracts the acidosis induced by starvation (Ziegler et al. 1992).

**Ornithine-α-ketoglutarate interactions**

By itself αKG has only limited effects on the liver. The increase in aspartate uptake and the absence of modification of NH₃ disposal in the αKG-treated group suggest that transamination reactions could be stimulated. This would explain the trend in the αKG-treated group towards increased glutamate release while glutamate content in the liver was high. However, we would have expected to observe a significant modification of glutamine, glutamate and NH₃ fluxes during an αKG load. It is possible that under our experimental conditions, i.e. relatively high perfusate glutamine concentration, αKG is preferentially oxidized. Indeed, Stoll & Haussinger (1989) observed in the perfused rat liver that more than 70% of αKG is oxidized when glutamine synthesis is inhibited.

We (Vaubourdolle et al. 1988; Cynober et al. 1990) and others (Winkler et al. 1993) have repeatedly demonstrated that αKG administration modifies Orn metabolism in humans and animals. For example, oral administration of OKG to healthy volunteers leads to an increase in plasma Arg, proline and glutamate. When the same subjects received either ornithine hydrochloride or αKG as its calcium salt, only plasma glutamate increased (Cynober et al. 1990). One line of evidence suggests that this metabolic interaction happens in the splanchnic area (Vaubourdolle et al. 1988). However, in the present study, only small changes were observed between the different groups. At most, a modification of proline metabolism in the OKG-perfused group could be observed. Thus, the increase in plasma proline after an OKG load (Cynober et al. 1990) could be due not only to the conversion of Orn into proline in the enterocyte (Vaubourdolle et al. 1988), but also to decreased hepatic uptake of proline.

To conclude, our findings indicate that while Orn and Arg are extensively metabolized by the liver, the catabolism of Arg is higher than that of Orn and leads to a higher increase in hepatic amino acid catabolism. Considering that a high liver metabolism is detrimental, it thus seems more logical to supply Orn rather than Arg to catabolic patients who exhibit an increase in N loss and a compromised acid-base homeostasis.

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