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Metabolism of Branched-Chain Amino Acids in Starved Rats: The Role of Hepatic Tissue

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Summary

Parameters of branched-chain amino acids (BCAA; leucine, isoleucine and valine) and protein metabolism were evaluated using L-[1-^{14}C]leucine and α-keto[1-^{14}C]isocaproate (KIC) in the whole body and in isolated perfused liver (IPL) of rats fed *ad libitum* or starved for 3 days. Starvation caused a significant increase in plasma BCAA levels and a decrease in leucine appearance from proteolysis, leucine incorporation into body proteins, leucine oxidation, leucine-oxidized fraction, and leucine clearance. Protein synthesis decreased significantly in skeletal muscle and the liver. There were no significant differences in leucine and KIC oxidation by IPL. In starved animals, a significant increase in net release of BCAA and tyrosine by IPL was observed, while the effect on other amino acids was non-significant. We conclude that the protein-sparing phase of uncomplicated starvation is associated with decreased whole-body proteolysis, protein synthesis, branched-chain amino acid (BCAA) oxidation, and BCAA clearance. The increase in plasma BCAA levels in starved animals results in part from decreased BCAA catabolism, particularly in heart and skeletal muscles, and from a net release of BCAA by the hepatic tissue.

Key words

Nutrition • Starvation • Leucine • Protein Metabolism • Branched-chain amino acids

Introduction

The branched-chain amino acids (BCAA), leucine, isoleucine and valine are indispensable amino acids of special interest. The BCAA are mainly used for protein synthesis and as a principal donor of nitrogen for *de novo* synthesis of alanine and glutamine in skeletal muscles. The initial step in BCAA catabolism occurs mainly in skeletal muscle because of a high activity of BCAA aminotransferase. Leucine, isoleucine, and valine are transaminated to form corresponding branched-chain keto acids (BCKA), α-ketoisocaproate (KIC), α-keto-β-methylvalerate, and α-ketoisovalerate, respectively. The BCKA products formed in this reaction then undergo oxidative decarboxylation catalyzed by BCKA dehydrogenase. Because of a low activity of BCKA dehydrogenase, skeletal muscle releases significant amounts of BCKA into the circulation (Odessey *et al.* 1974). It should be noted that human muscles contain 60 % of the total BCKA dehydrogenase, while rat muscle

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contains 10-30 %. This is mainly because the BCKA dehydrogenase activity is low in the human liver. Although a greater proportion of BCKA may be oxidized in human than in rat muscle, BCKA are released significantly from human muscle and circulate in the blood (Harper *et al.* 1984). The BCKA released into the blood stream are then oxidized in tissues where BCKA dehydrogenase in its active form is found in abundance or used for the resynthesis of BCAA (Randle *et al.* 1984, Harper *et al.* 1984).

The ability of tissues to reaminate BCKA and release the respective amino acids has been shown in the brain, heart, liver, kidney, and skeletal muscle (Harper *et al.* 1984). Abumrad *et al.* (1982) used postabsorptive dogs with implanted venous catheters and demonstrated that after infusion of KIC into the gut, roughly 59 % of the absorbed KIC was taken up by the liver and one-third of this was transaminated to leucine. Matthews *et al.* (1981) demonstrated in postabsorptive humans that the rate of irreversible loss of leucine (oxidation to KIC) was only 9 % of the leucine flux, while reamination of KIC to leucine represented 91 % of the flux. These results suggest that cycling of BCAA carbon skeleton *via* transamination-reamination is much more extensive than irreversible oxidation.

Enhanced rates of BCAA oxidation have been observed in the whole body and in skeletal muscle during sepsis, trauma and after endotoxin or tumor necrosis factor treatment (Nawabi *et al.* 1990, Holeček 1996, Holeček *et al.* 1997). In the recent study, we observed decreased activity of BCKA dehydrogenase in skeletal muscles and the heart of rats starved for 2 or 4 days while starvation for 6 days caused a marked activation of the enzyme (Holeček 2001). The decreased BCKA dehydrogenase activity indicates decreased oxidation of BCAA and is undoubtedly a part of the adaptive response of the body which prevents rapid development of protein wasting. However, considering the differences of individual tissues in BCAA metabolism (Harper *et al.* 1984, Randle *et al.* 1984), the impact of starvation on BCAA metabolism in individual tissues may be different. It should be noted that the loss of tissue proteins during dietary restriction is more pronounced in the splanchnic region, such as the liver and small intestine, whereas skeletal muscle proteins are less affected (Holeček *et al.* 1995, Felgines *et al.* 1999). The present study was undertaken to evaluate the changes of BCAA catabolism in the whole body and the liver during the protein-sparing phase of starvation.

Methods

Animals

Male Wistar rats (Velaz, Prague, CR) were housed in standardized cages with controlled temperature and a 12-hour light-dark cycle and received Velaz-Altromin 1320 laboratory chow and drinking water *ad libitum*. All procedures involving animals were performed according to the guidelines set by the Institutional Animal Use and Care Committee of Charles University.

Materials

L-[1-^{14}C]leucine and α-keto[1-^{14}C]isocaproate were purchased from Amersham (Buckinghamshire, UK), [^{14}C]bicarbonate was obtained from Du Pont-NEN (Bad Homburg, Germany). Leucine and the sodium salt of α-ketoisocaproate were purchased from Sigma (St. Louis, MO). Amino acid solution Amino-Mel 10 % was obtained from Fresenius (Germany). Hyamine hydroxide was obtained from Packard Instrument (Meriden, CT). The remaining chemicals were obtained from Lachema (Brno, CR).

Experimental design

Two separate studies evaluating the effect of short-term starvation on BCAA metabolism were performed. The duration of starvation (3 days) was determined on the basis of our previous study in which we demonstrated a significant decrease in BCKA dehydrogenase activity in rats starved for 2 or 4 days (Holeček 2001).

Study 1: Effect of starvation on whole-body protein and BCAA metabolism

A polyethylene cannula was inserted into the jugular vein 24 h before the experiment to avoid the effect of surgery. Rats fed *ad libitum* or rats starved for 3 days were placed in a glass metabolic cage and infused with L-[1-^{14}C]leucine (1.9 μCi/ml). A priming dose of 0.7 ml (i.e. 1.33 μCi of L-[1-^{14}C]leucine) was followed by a constant infusion at a rate 0.36 ml/h for 200 min. In the *ad libitum* fed group, food was removed 3 h before the infusion of the tracer to avoid the effect of food digestion and absorption. The rats were killed by exsanguination *via* the abdominal aorta exactly at the 321st min from the beginning of the infusion.

The parameters of whole-body leucine metabolism were evaluated under steady-state conditions

by the procedure described in detail previously (Holeček *et al.* 1997). The expired CO_2 was trapped by monoethanolamine at 10-min intervals between the 125th and 185th min of infusion. The average value of six measurements of $^{14}CO_2$ radioactivity in expired air under steady-state condition was used for calculating the leucine oxidation rate. The $^{14}CO_2$ recovery factor (RF) estimated by infusion of [^{14}C] bicarbonate was about 90 % in both control and experimental animals. Leucine specific activity (SA_{Leu}), turnover (Q_{Leu}), clearance (C_{Leu}) and decarboxylation (D_{Leu}) rates were calculated by the following formulas:

$$SA_{Leu}\,(dpm \cdot \mu mol^{-1}) = \frac{\text{Leu radioactivity } (dpm \cdot ml^{-1})}{\text{Leu concentration } (\mu mol \cdot ml^{-1})}$$

$$Q_{Leu}\,(\mu mol \cdot h^{-1}) = \frac{\text{infusion rate } (dpm \cdot h^{-1})}{SA_{Leu} \text{ in plasma } (dpm \cdot \mu mol^{-1})}$$

$$C_{Leu}\,(ml \cdot h^{-1}) = \frac{Q_{Leu}\,(\mu mol \cdot h^{-1})}{\text{plasma Leu } (\mu mol \cdot ml^{-1})}$$

$$D_{Leu}\,(\mu mol \cdot h^{-1}) = \frac{^{14}CO_2 \text{ production rate } (dpm \cdot h^{-1})}{SA_{Leu} \text{ in plasma } (dpm \cdot \mu mol^{-1}) \times RF}$$

Whole-body leucine metabolism was considered to take place within a common metabolic pool represented by free plasma leucine. Because exogenous leucine intake (E) was zero in our protocol, Q_{Leu} estimates the leucine released from protein, i.e. the protein breakdown (B) as described by the equation:

$$Q = In + D = B + E.$$

Using this formula, rates of leucine incorporation in body proteins (In) and the oxidized fraction of leucine (OF = D · 100/Q) were calculated.

Samples of gastrocnemius muscle and liver were immediately frozen in liquid nitrogen and used for measurement of protein synthesis as described elsewhere (Holeček *et al.* 1997). The protein content was measured according to Lowry *et al.* (1951).

Study 2: The effect of starvation on BCAA metabolism in the isolated perfused liver (IPL)

Leucine and KIC oxidation were estimated using the single-pass perfusion technique (Patel *et al.* 1981). *Ad libitum*-fed or 3-day-starved rats were anesthetized with sodium pentobarbital (35 mg · kg b.w.$^{-1}$ intraperitoneally) and the livers prepared for perfusion as described in detail elsewhere (Holeček *et al.* 1996). Briefly, the bile duct was cannulated after laparotomy and 1000 IU · kg^{-1} of heparin were injected into the saphenous vein. Then the portal vein was cannulated with a polyethylene catheter (ID 1.5 mm) and the hepatic artery was ligated. During portal perfusion with Krebs-Henseleit solution (20 °C) the liver was quickly removed. The perfusion was carried out at 37 °C in a thermostatically controlled cabinet. A peristaltic pump passed the perfusate from the reservoir through an oxygenator and a bubble trap into the liver. The flow rates were maintained at 3.5 ml · g liver^{-1} · min^{-1}. The membrane oxygenator was made of thin-walled silicone tubing (Silastic; i.d. 0.058 in, o.d. 0.077 in) 25 ft in length, enclosed in a glass cylinder continually gassed with a mixture of O_2 and CO_2 (95 % : 5 %) at a flow rate of 500 ml · min^{-1}. The glass bubble trap also served as a peristaltic wave compensator. The viability of perfused livers was monitored by their appearance, concentration of minerals and liver enzymes in the perfusate, and by the stability of bile flow.

The perfusion medium consisted of Krebs-Henseleit bicarbonate buffer, 10 mM glucose and amino acids at about the normal plasma concentrations in rats (996 ml Krebs-Henseleit solution mixed with 4 ml Amino-Mel 10 %, pH 7.4). Adding amino acids in the perfusion solution prevents excessive proteolysis (De Bandt *et al.* 1990). The perfusion solution for measuring KIC oxidation contained 1.26 mM leucine, 1 mM KIC and α-keto[1-^{14}C]isocaproate (2 μCi · l^{-1}) as a tracer (Single pass No. I). The perfusion solution for leucine oxidation estimation contained 1.26 mM leucine, and [1-^{14}C]leucine (10 μCi · l^{-1}) as tracer (Single pass No. II). In agreement with other studies (Patel *et al.* 1981, Blonde-Cynober *et al.* 1995), our preliminary experiments showed that 1 mM KIC and 1 mM leucine were necessary for the substrate to be non-limiting during the flux study.

Prior to starting the experimental protocol, livers were perfused with a tracer-free perfusion medium for a period of 10 min to ensure stabilization of the liver and washout of endogenous hormones. In other studies we observed a marked decrease in portal resistance between 5 and 10 min intervals, but there was no substantial difference between 10 min and 15 min resistance values (Tilšer *et al.* 1996). At the 11th minute, the perfusion medium containing labeled KIC was infused for 15 min. Then the livers were perfused with a tracer-free medium

for 5 min and at the 31st min the perfusion medium containing labeled leucine was infused for 15 min. Samples of the effluent perfusate were collected in 20 ml flasks equipped with stoppers and center wells containing 0.4 ml of methylbenzethonium hydroxide at 1 min intervals to monitor $^{14}CO_2$ production. Labeled CO_2 in the perfusate, which was produced from the infused 1-^{14}C-labeled substrates, was released by injecting 0.5 ml of 5 N sulfuric acid through the stopper into the flasks. Oxidation rates of KIC and leucine were calculated as follows:

$$O = (R \cdot F \cdot RF) / (SA \cdot W)$$

where O is the substrate oxidation (μmoles of substrate oxidized · g of dry liver^{-1} · h^{-1}), R is the radioactivity of $^{14}CO_2$ in the effluent perfusate (dpm · ml^{-1}), F is the flow rate of perfusion medium through the liver (ml · h^{-1}), RF is the recovery factor, SA is the specific activity of KIC or leucine in the perfusion medium (dpm · μmol^{-1}) and W is the liver dry weight in grams. The $^{14}CO_2$ recovery was 97±0.6 % in control and 98±0.9 % in starved rats, respectively. Thus the same correction factor (0.98) was used for each group of rats.

The single pass phase of the study was followed at the 46th minute by a recirculation phase (15 min) in which we measured the uptake and release of individual amino acids by IPL. Amino acid exchanges were calculated as follows:

$$E = (C_{t60} - C_{t46}) \cdot V/(W_{dry} \cdot t)$$

where C_{t60} and C_{t46} are amino acid concentrations at the end and at the beginning of the recirculation phase of the study, V is the total volume of perfusate in liters, t is the duration of recirculation in hours and W_{dry} is the liver dry weight in grams. The results are expressed as μmol · g dry liver^{-1}· h^{-1}. Negative values mean net amino acid uptake, positive values indicate net release.

Other techniques

Amino acid concentrations were determined by high-performance liquid chromatography (Waters, Milford, MA) after precolumn derivatization with *o*-phthaldialdehyde. The radioactivity of the samples was measured with a liquid scintillation radioactivity counter LS 6000 (Beckman Instruments, Fullerton, CA). Glucose concentration and activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using commercial tests (Lachema, Brno, CZ). Na^+ and K^+ were determined using ion-selective electrodes on AVL 983-S (Austria).

Statistical analysis

The results are expressed as means ± S.E.M. Statistical analysis was performed by the F-test and Student's t-test. A difference was considered significant at $P<0.05$. Statistical software NCSS60 was used for the analysis.

Table 1. Effect of starvation on body weight and whole-body leucine metabolism *(Study 1)*.

	Control (n=6)	**Starvation 3 days (n = 7)**
Body weight - before (g)	229 ± 3	308 ± 3*
- after (g)	245 ± 3	259 ± 3*
- change (%)	6.9 ± 1.1	- 16.0 ± 0.5*
Leucine incorporation in proteins (μmol/kg/h)	159 ± 12	116 ± 8*
Leucine appearance from proteolysis (μmol/kg/h)	197 ± 14	134 ± 9*
Leucine oxidation (μmol/kg/h)	38.1 ± 2.3	18.0 ± 1.6*
Leucine oxidized fraction (%)	19.5 ± 0.5	13.5 ± 0.9*
Leucine clearance (ml/kg/h)	1194 ± 69	722 ± 21*

*Parameters of leucine metabolism were evaluated in the whole body using intravenous infusion of [1-^{14}C]leucine. Mean ± S.E.M. *P < 0.05 vs. control.*

Results

Study 1: Effect of starvation on whole-body protein and BCAA metabolism.

At the beginning of the experiment we had rats of a lower body weight in the control group than in the experimental one as we have been trying to reach approximately the same body weight at the end of the study. Nevertheless there was a slight difference in body weights between control and starved animals on the third day of experiment (Table 1).

Table 2. Effect of starvation on protein concentration and protein synthesis in skeletal muscle and liver *(Study 1).*

	Control (n = 6)	Starvation 3 days (n = 7)
Muscle		
- protein	130 ± 7	134 ± 4
- protein synthesis	0.71 ± 0.05	0.30 ± 0.03*
Liver		
- protein	106 ± 1	120 ± 3*
- protein synthesis	2.70 ± 0.21	1.65 ± 0.09*

*Protein concentration is given in mg/g. Protein synthesis (nmol Leu/mg/h) was estimated on the basis of [1-^{14}C]leucine incorporation in protein and [1-^{14}C]leucine specific activity in the tissue.. *P < 0.05 vs. control.*

Starvation for 3 days caused a marked decrease in whole-body leucine turnover which is demonstrated by a significant decrease in leucine incorporation into proteins (indicator of whole-body protein synthesis), leucine appearance from proteolysis (indicator of protein breakdown), leucine oxidation, leucine oxidized fraction and leucine clearance (Table 1). The increase in hepatic protein concentration in starved animals is caused undoubtedly by a decrease in glycogen content. Protein synthesis decreased significantly in both skeletal muscles and the liver (Table 2). A significant increase in plasma levels of serine, glycine, isoleucine, tyrosine, phenylalanine, arginine and BCAA was observed in starved animals while glutamine, alanine, citrulline and ornithine levels were decreased (Table 3).

Table 3. Effect of starvation on plasma amino acid levels *(Study 1).*

	Control (n = 6)	Starvation 3 days (n = 7)
Taurine	138 ± 16	152 ± 23
Aspartate	23 ± 4	24 ± 2
Threonine	136 ± 6	138 ± 6
Serine	121 ± 11	160 ± 3*
Asparagine	29 ± 4	32 ± 3
Glutamate	122 ± 11	152 ± 10
Glutamine	621 ± 45	488 ± 22*
Proline	98 ± 9	92 ± 5
Glycine	186 ± 26	381 ± 22*
Alanine	267 ± 24	176 ± 19*
Citrulline	57 ± 7	37 ± 2*
Valine	160 ± 13	172 ± 19
Methionine	28 ± 4	33 ± 2
Isoleucine	56 ± 5	74 ± 3*
Leucine	164 ± 7	185 ± 11
Tyrosine	29 ± 3	39 ± 2 *
Phenylalanine	42 ± 4	60 ± 2 *
Tryptophan	15 ± 2	21 ± 3
Ornithine	17 ± 1	6 ± 1*
Lysine	171 ± 23	211 ± 15
Histidine	38 ± 3	34 ± 2
Arginine	61 ± 7	100 ± 4*
Derived values		
BCAA	381 ± 13	431 ± 17*
Total amino acids	2581±126	2764 ± 63

*Units are μmol · l^{-1}. *P< 0.05 vs. control.*

Study 2: The effect of starvation on BCAA metabolism in the isolated perfused liver (IPL).

Although the liver weight of starved animals was significantly lower than those of fed rats, there were no differences in the dry-to-wet weight ratio, in the bile flow or in glucose, ALT, AST, potassium and sodium concentrations in perfusate at the end of the recirculation phase (Table 4). There were no significant differences in KIC and leucine oxidation by IPL (Figs 1 and 2).

The recirculation phase of the experiment showed a net uptake of all estimated amino acids in control animals. In the starved animals, a significant decrease in net uptake of tyrosine and release of BCAA was observed in comparison with the controls (Table 5).

Table 4. Parameters of isolated perfused livers of control and starved animals *(Study 2).*

	Control (n = 5)	Starvation 3 days (n = 6)
Wet LW (g/kg b.w.)	32.9 ± 2.5	23.9 ± 0.6*
Dry LW (g/kg b.w.)	9.7 ± 0.6	7.5 ± 0.4*
Dry/Wet (%)	29.4 ± 0.7	31.4 ± 1.3
Bile flow (mg/g/h)	144 ± 16	105 ± 15
Glucose (mmol/l)	10.2 ± 0.1	9.7 ± 0.2
AST (μkat/l)	0.09 ± 0.02	0.16 ± 0.05
ALT (μkat/l)	0.12 ± 0.03	0.11 ± 0.02
Na^+ (mmol/l)	139 ± 1	141 ± 1
K^+ (mmol/l)	5.2 ± 0.1	5.3 ± 0.0

*Glucose, ALT, AST, potassium and sodium concentrations were measured in the perfusate at the end of the recirculation phase (15 min). LW – liver weight. *P<0.05 vs. control.*

Table 5. Effect of starvation on net amino acid uptake (–) or release (+) by isolated perfused livers *(Study 2).*

	Control (n = 5)	Starvation 3 days (n = 6)
Aspartate (230)	–5.3 ± 0.9	–3.9 ± 1.3
Threonine (134)	–4.0 ± 1.3	–3.1 ± 0.8
Serine (350)	–7.2 ± 2.5	–6.3 ± 2.6
Glycine (362)	–5.4 ± 1.3	–2.3 ± 1.6
Alanine (350)	–13.8 ± 2.3	–15.9 ± 2.6
Valine (191)	–2.8 ± 0.3	–2.0 ± 0.8
Methionine (107)	–3.3 ± 0.2	–2.8 ± 0.4
Isoleucine (134)	–0.1 ± 1.5	+0.7 ± 0.2
Leucine (262)	–1.8 ± 0.7	+1.8 ± 1.1*
Tyrosine (181)	–7.0 ± 0.9	–1.6 ± 0.7*
Phenylalanine (111)	–3.2 ± 0.4	–2.4 ± 0.5
Tryptophan (35)	–0.7 ± 0.0	–0.5 ± 0.1
Lysine (157)	–2.8 ± 0.5	–4.1 ± 0.7
Histidine (113)	–3.2 ± 0.4	–3.7 ± 0.7
Derived values		
BCAA (587)	–4.8 ± 1.3	+0.5 ± 1.5 *
Total AA (2,717)	–60.8 ± 2.8	–46.1 ± 3.5*

*The rates of amino acid exchange were estimated using IPL during the recirculation phase of the study. For details see "Methods". Units are μmol · g dry liver^{-1} · h^{-1}. *P<0.05 vs. control. Values in parentheses (first column) indicate amino acid concentrations in perfusion solution in μmol/l at the beginning of perfusion.*

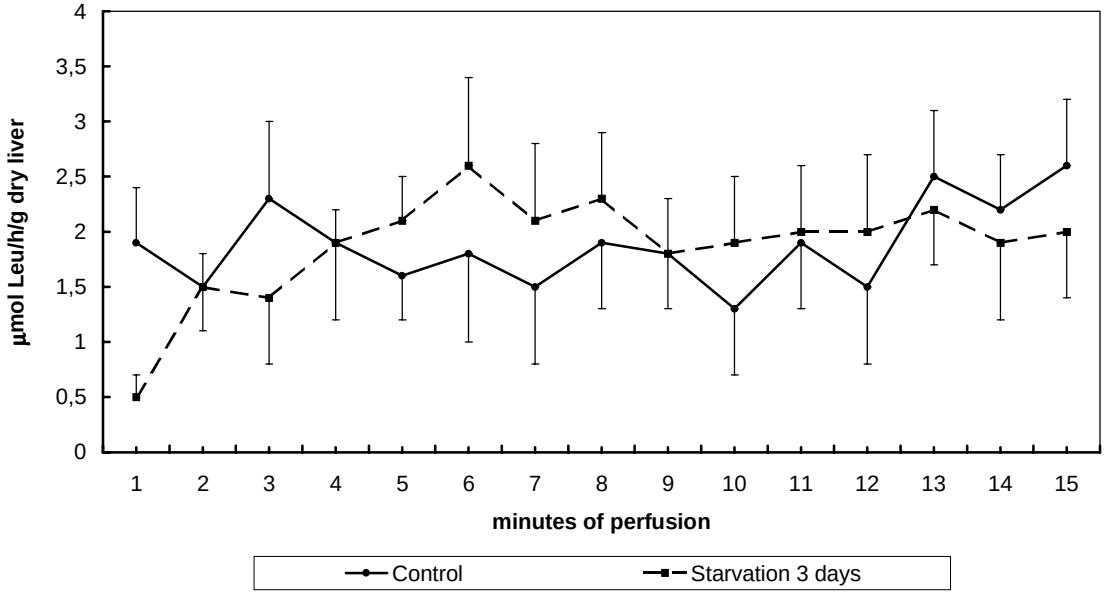

Fig. 1. *The effect of starvation (3 days) on leucine oxidation by isolated perfused livers. The flux through liver BCKA dehydrogenase was estimated using the single-pass perfusion technique with [1-^{14}C]leucine as a tracer. Data are means ± S.E.M.*

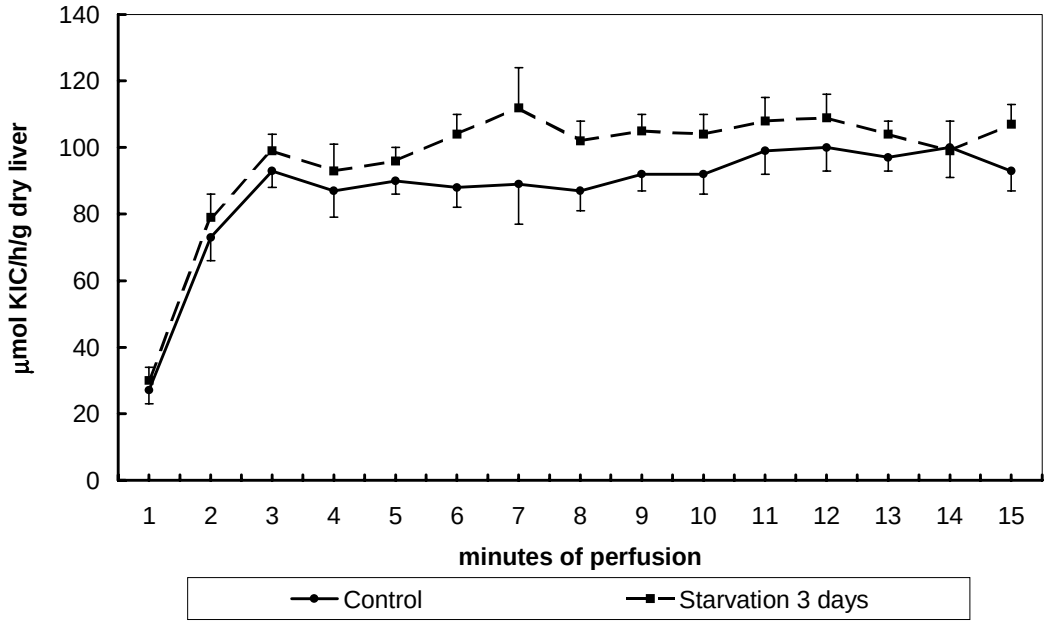

Fig. 2. *The effect of starvation (3 days) on KIC oxidation by isolated perfused livers. The flux through liver BCKA dehydrogenase was estimated using the single-pass perfusion technique with α-keto[1-^{14}C]isocaproate as a tracer.*

Discussion

Starvation of the rat for three days corresponds to the phase of protein sparing, in which the main energy fuels are lipids and ketone bodies, whereas glycerol, lactate and pyruvate are the principal substrates for gluconeogenesis (Cahill 1970, Goodman *et al.* 1980). In this phase of starvation, a marked decrease of myofibrillar protein degradation was recorded (Lowell *et al.* 1986), together with decreased amino acid uptake in skeletal muscle (Warner *et al.* 1989), and decreased activity of BCKA dehydrogenase, the key enzyme in BCAA oxidation in muscles and the heart (Holeček 2001). The decrease in whole-body leucine appearance from proteolysis and decreased rate of leucine oxidation observed in this study clearly demonstrate an important response of the body which prevents the loss of essential BCAA and wasting of the body proteins. Similar changes in protein turnover were also demonstrated in rats after 112 h of starvation when L-[2,6-^{3}H]phenylalanine was used as a tracer (De Blaauw *et al.* 1996). The decrease in leucine clearance during starvation, which undoubtedly results from decreased incorporation of leucine in body proteins and leucine oxidation, can explain greater increments in plasma leucine concentration after leucine infusion during fasting than in the postabsorptive state (Sherwin 1978).

The rise in plasma BCAA levels during starvation observed in this study is in agreement with the results of other laboratories (Hutson and Harper 1981, Sherwin 1978, Ahlman *et al.* 1994). However, the mechanism underlying the initial rise and subsequent decline in plasma BCAA during starvation is still not clear yet. The decreased protein synthesis rate cannot be a sufficient explanation, as the increase in BCAA levels during starvation is not accompanied by increased concentrations of other amino acids. The responsible factor should be changes in BCAA oxidation or alterations in the interconversion of BCAA and BCKA (transamination). The decreased activity of the key enzyme in the catabolism of BCAA, i.e. BCKA dehydrogenase, which should significantly affect the conversion of BCKA to BCAA and thus be a cause of a specific increase in BCAA levels in muscle and body fluids, was observed in skeletal muscle and the heart (Holeček 2001). The role of decreased activity of BCKA dehydrogenase in skeletal muscle in the pathogenesis of increased plasma BCAA levels during starvation is supported by observations of a higher increase of BCAA levels in muscles than in the plasma or other tissues during starvation (Hutson and Harper 1981).

The BCKA generated in the BCAA aminotransferase reaction in skeletal muscles are believed to be released significantly into the blood stream and

taken up mainly by the liver, where BCKA dehydrogenase in the active form is abundant (Harper *et al.* 1984). For this reason, an important role of hepatic tissue in the catabolism of BCAA under different conditions can be suggested. In the present study, neither significant effect of starvation on the flux of leucine through hepatic BCAA aminotranferase nor KIC through hepatic BCKA dehydrogenase have been observed. Similarly, Harris *et al.* (1985) demonstrated no effect of starvation for 48 h in rats on the activity state of hepatic BCKA dehydrogenase. Nevertheless, higher concentrations of BCAA in the effluate from livers of starved animals than in the effluate of hepatic tissue of control animals (Table 3) indicate a higher release of BCAA by the liver of starved animals. This net release of BCAA can be caused by their decreased utilization or increased release. Considering the decreased whole-body leucine turnover, leucine clearance and leucine incorporation into liver proteins of starved rats (Study 1), the net efflux of BCAA from the liver should be related to the decreased uptake of BCAA by hepatic tissue, a more significant decrease in protein synthesis than in proteolysis, and/or to lower hepatic catabolism of BCAA than of other amino acids.

In conclusion, the results of the present study demonstrate that the protein sparing phase of uncomplicated starvation is associated with decreased whole-body proteolysis, protein synthesis, BCAA oxidation, and BCAA clearance. The increase in plasma BCAA levels is related to decreased BCAA catabolism, particularly in skeletal muscle and heart as demonstrated recently (Holeček 2001), and to the net release of BCAA from hepatic tissue.

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