Biochem. J. (1991) 276, 405–409 (Printed in Great Britain)

Leucine and glutamine metabolism in septic rats

Shogo YOSHIDA,* Susan LANZA-JACOBY† and T. Peter STEIN*‡

*Departments of Surgery, University of Medicine and Dentistry of New Jersey, School of Osteopathic Medicine, Camden, NJ 08103, and †Thomas Jefferson University, Philadelphia, PA 19128, U.S.A.

The rate of leucine C-2 incorporation into glutamine was compared in control and septic rats. Female Sprague–Dawley rats ($n = 46$, 210–260 g) were fed parenterally for 3 days and then randomized into two groups (control and septic). Sepsis was induced by the injection of 10^{10} live *Escherichia coli*/kg on day 4 into the septic group. Rats in each group were given a continuous (8 h) infusion of one of three different isotopes. The isotopes were given 24 h after inoculation. Leucine oxidation and incorporation into protein were determined with [1-^{13}C]leucine; glutamine flux and oxidation were determined with [5-^{13}C]glutamine, and the fraction of leucine C-2 incorporated into glutamine was determined by giving [1,2-^{13}C]leucine. Results were as follows: sepsis caused a significant increase in the rate of leucine C-2 incorporation into glutamine (66.0 ± 3.7 as against 29.6 ± 3.7 μmol/h per kg, $P < 0.01$). This increase was due to both an increase in glutamine production (2331 ± 76 as against 1959 ± 94 μmol/h per kg, $P < 0.01$) and an increase in the proportion of glutamine derived from leucine (2.83 ± 0.27 % as against 1.51 ± 0.31 %, $P < 0.01$). The ratio of leucine C-2 incorporated into glutamine to leucine oxidized increased from 7.16 ± 0.91 % to 11.49 ± 1.12 % with sepsis ($P < 0.05$).

INTRODUCTION

Sepsis induces profound changes in whole-body amino acid metabolism. Prominent among the changes are increased proteolysis, glutamine production, leucine production and leucine oxidation (Blackburn *et al.*, 1979; Newsholme *et al.*, 1988; Rennie *et al.*, 1989; Wolfe *et al.*, 1989). The additional glutamine is used as a fuel and a biosynthetic substrate for the increased lymphocytic activity that occurs after sepsis or injury (Newsholme *et al.*, 1988). The amino N from leucine and the other two branched-chain amino acids serves as precursors for glutamine N, and the oxidation of the carbon skeletons provide energy for muscle (Hagenfeldt *et al.*, 1980; Aoki *et al.*, 1981; Lund & Williamson, 1985). It is believed that the metabolic fate of leucine carbon atoms is limited to incorporation into protein or oxidation (Odessey *et al.*, 1974; Melville *et al.*, 1989).

However, in severely stressed states this assumption may not always be the case. The insulin resistance which occurs during stress states restricts glucose uptake by muscle (Black *et al.*, 1982). One consequence of the diminished glucose supply is increased branched-chain-amino-acid oxidation by muscle (Blackburn *et al.*, 1979). Another potential consequence is that a greater proportion of the precursors of muscle originated metabolites (e.g. glutamine) will have to be derived from intracellular sources such as leucine, since the supply of two- and three-carbon precursors from the plasma is likely to be restricted.

Thus it is possible, in stressed states such as sepsis, that leucine carbon atoms can contribute to glutamine synthesis via the muscle two-carbon pool and the α-oxoglutarate derived from it. In the present study we determined whether leucine C-2 catabolism via incorporation into glutamine is a significant pathway for leucine carbon metabolism in the septic state.

The rate of leucine C-2 incorporation into plasma glutamine was determined in septic and control rats. Three separate isotopic tracers were used. Leucine oxidation and incorporation into protein was determined by giving [1-^{13}C]leucine and measuring the ^{13}C enrichment of the expired CO_2. The glutamine flux was determined by giving [5-^{13}C]glutamine and calculating the glutamine flux from the plasma glutamine enrichment. The proportion of the plasma glutamine flux derived from leucine C-2 was determined by giving [1,2-^{13}C]leucine and measuring the incorporation of ^{13}C from leucine C-2 into plasma glutamine.

MATERIALS AND METHODS

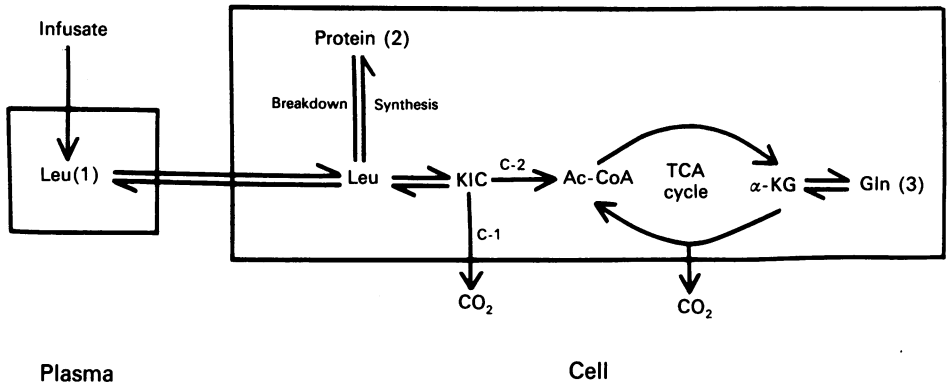
Female Sprague–Dawley rats ($n = 46$, 210–260 g) were fed a stock rat diet (5001 Lab Chow C; Purina, Richmond, IN, U.S.A.) *ad lib* until the time of study, with a 12 h-light/12 h-dark regime. The rats were anaesthetized with a Ketamine/Acepromazine mixture (75/7.5 mg/kg) and a catheter inserted for intravenous feeding as previously described (Stein *et al.*, 1981 *a*,*b*). After the surgery the rats were placed in individual metabolic cages. Half-strength total parenteral nutrition was infused on day 1 [amino acids (AA): 523 kJ (125 kcal)/day per kg and 750 mg of N/day per kg], 4/5 strength on day 2 [837 kJ (200 kcal)/day per kg and 1200 mg of N/day per kg] and full strength on days 3 and 4 [1046 kJ (250 kcal)/day per kg and 1500 mg of N/day per kg]. The energy (cal)/N ratio was 166:1 [energy (J)/N ratio 698:1]. The diet was made using 70% Dextrose, Freamine III, 20% Nutrilipid and appropriate vitamins and minerals (Kendall-McGaw, Irvine, CA, U.S.A.). The animals were randomized into two groups (control and septic). Sepsis was induced by the injection of 10^{10} live *Escherichia*/kg through the catheter on day 4. After 16 h (day 5), an 8 h constant infusion of each isotope was started for both septic and control rats.

Rats in each group were given one of three different isotope infusates: (1) [1,2-^{13}C]leucine (4 mg/h; Tracer Technologies, Cambridge, MA, U.S.A.) to determine leucine flux and what percentage of glutamine was derived from leucine; (2) [5-^{13}C]glutamine (2 mg/h; Merck Frosst Canada, Montreal, Canada) to determine glutamine flux and glutamine oxidation; (3) [1-^{13}C]leucine (4 mg/h; Tracer Technologies) to determine leucine oxidation and what percentage of leucine was oxidized via glutamine.

The isotopes were all given in full-strength diet [1046 kJ (250 kcal)/day per kg, 1500 mg of N/day per kg] as an 8 h

Abbreviations used: KIC, 'α-ketoisocaproate' (α-oxoisohexanoate); APE, atom percentage excess.

‡ To whom correspondence should be sent.



Scheme 1. Pathway of incorporation of leucine C-2 into glutamine

Abbreviations: Ac, acetyl; α-KG, 'α-ketoglutarate' (α-oxoglutarate); TCA, tricarboxylic acid.

Table 1. [1-^{13}C]Leucine kinetics

d is the isotope-infusion rate, Leu_{APE} and KIC_{APE} are the ^{13}C enrichments of leucine and KIC in atom percentage excess (APE), $R_{a,Leu}$ and $R_{a,21}$ are the total and endogenous rates of appearance of leucine in the plasma, $Leu_{ox.}$ the rate of leucine oxidation and PSR is the rate of protein synthesis. Statistics in this and the following Tables were two tailed *t*-tests (ns, not significant).

Parameter	Value		
	Control	Sepsis	$P <$
No. of rats (*n*)	6	6	
Weight of rats (g)	244±14	241±15	ns
d (μmol/h per kg)	134±6.6	139±12	ns
Infusion vol. (ml/h)	2.14±0.08	2.16±0.12	ns
Leu_{APE} (%)	17.90±1.32	15.42±0.75	ns
KIC_{APE} (%)	13.20±0.88	9.79±0.79	0.05
$R_{a,Leu}$ (μmol/h per kg)	628±39	758±25	0.05
$R_{a,21}$ (μmol/h per kg)	246±45	363±22	0.05
$Leu_{ox.}$ (μmol of Leu/h per kg)	413±20	574±66	0.05
PSR (μmol of Leu/h per kg)	215±27	184±60	ns

continuous infusion. Blood samples were collected after decapitation, the plasma being separated by centrifugation (3000 *g*, 15 min), and stored at −20 °C until analysis. To measure leucine and glutamine oxidation, the animals were placed in glass metabolic cages (Bellco Glass, Vineland, NJ, U.S.A.) before beginning the isotope infusions. Expired air was bubbled through two flasks, each containing 75 ml of 0.2 M-NaOH, to trap expired CO_2 for 1 h before (background) and for 1 h at the end of isotope infusion. Ascarite II (A. H. Thomas, Swedesboro, NJ, U.S.A.) was used to remove CO_2 from the air before entry into the cage. $^{13}CO_2$ enrichment was determined by m.s. performed by Global Geochemistry (Canoga Park, CA, U.S.A.).

Glutamine was separated from glutamate as described by Darmaun *et al.* (1985). Plasma (0.5 ml) was deproteinized with sulphosalicyclic acid (10 %, w/w; 0.5 ml) and centrifuged (3000 *g*, 15 min). The supernatant was neutralized with 1 M-KOH (about nine drops) and combined with pH 9 buffer (5 ml; Fisher Scientific, Fairlawn, NJ, U.S.A.). The solution was then applied to an anion-exchange column containing 1.5 g of AG1-X8 resin (formate form; 200–400 mesh; Bio-Rad Laboratories, Richmond, CA, U.S.A.). The eluate was acidified with a few drops of 3 M-HCl and applied to a cation-exchange column containing 1 g of Dowex 50W-X8 resin (H^+ form; 200–400 mesh; Sigma, St. Louis, MO, U.S.A.). The column was washed with water (10 ml) and the glutamine was eluted with aq. 2 M-NH_3 (3 ml), followed by water (2 ml). The NH_3 was removed under a stream of N_2 in a hot-water bath (until half volume was reached), and the remaining liquid was freeze-dried. The glutamine was then converted into its *N*-acetyl-*N*-propyl derivative for g.c.–m.s. analysis as previously described (Stein *et al.*, 1986). The derivative was injected into either a Hewlett–Packard 5970 or a 5992A gas chromatograph/mass spectrometer in the selected ion-monitoring mode. The mass fragments at *m/z* 144 and 145 were monitored.

Leucine was co-eluted from the Dowex 50-X8 with the glutamine, and thus it was derivatized at the same time. The fragments at *m/z* 99, 100, 101 were monitored according to whether the tracer used was [1-^{13}C]leucine (99 and 100) or [1,2-^{13}C]leucine (99 and 101).

'α-Ketoisocaproate' (KIC; α-oxoisohexanoate) was isolated from plasma (0.5 ml) by a modification of the method described by Wolfe (1984). The plasma was deproteinized with acetone at 0 °C, and the precipitate was removed by centrifugation for 10 min at 8000 *g*. After drying the supernatant under N_2 at 50 °C, the residue was dissolved in 0.01 M-NaH_2PO_2 (0.5 ml) and chromatographed on a C_{18} silica-gel column. The KIC-containing fractions were freeze-dried and derivatized with *NO*-bis(trimethyl) trifluoracetate (0.1 ml; Sigma) and pyridine (0.15 ml) at 60 °C for 20 min and then injected into the g.c.–m.s. instrument. The fragments at *m/z* 259 and 260 were monitored for [1-^{13}C]KIC and at *m/z* 259 and 261 for [1,2-^{13}C]KIC.

METHODS OF CALCULATION

Scheme 1 shows the pathway for leucine C-2 incorporation into glutamine.

Protein synthesis and breakdown

The total leucine appearance rate in the plasma ($R_{a,Leu}$) was calculated from eqn. (1). *d* is the isotope infusion rate, $Leu_{inf.}$ and Leu_{APE} are the enrichments of the stock labelled leucine and the plasma leucine in atom percentage excess (APE; Wolfe, 1984).

$$R_{a,Leu} = d \cdot (Leu_{inf.}/Leu_{APE}) \quad (1)$$

The endogenous leucine production rate ($R_{a,21}$; ~ protein breakdown) rate was calculated by subtracting the rate of

exogenous leucine infusion from $R_{a,Leu}$. Breakdown rates were calculated from both the [1-^{13}C]- and the [1,2-^{13}C]-leucine data. The protein-synthesis rate (PSR) was calculated from eqns. (2) and (3) by using the [1-^{13}C]leucine data. $Leu_{ox.}$ is the rate of leucine oxidation, $CO_{2,APE}$ the enrichment of breath CO_2, V_{CO_2} the rate of CO_2 production, KIC_{APE} the plasma [1-^{13}C]KIC enrichment and 0.9 the correction factor for metabolic sequestration of CO_2 (Wolfe, 1984).

$$Leu_{ox.} = (CO_{2,APE} \cdot V_{CO_2}/(KIC_{APE} \cdot 0.9) \quad (2)$$

$$R_{a,Leu} = PSR + Leu_{ox.} \quad (3)$$

Calculation of the fraction of leucine C_2 transferred to glutamine

The fraction of the glutamine flux derived from leucine (F_{Gln}) was calculated from eqn. (4). $Gln_{1,2Leu\text{-}APE}$ is the enrichment of plasma glutamine when [1,2-^{13}C]leucine is given, and KIC'_{APE} is the plasma [1,2-^{13}C]KIC enrichment.

$$F_{Gln} = Gln_{1,2\,Leu\text{-}APE}/KIC'_{APE} \quad (4)$$

The rate of endogenous glutamine appearance in the plasma ($R_{a,Gln}$), was calculated from the enrichment of the stock labelled glutamine ($Gln_{inf.}$), plasma glutamine ^{13}C enrichment (Gln_{APE}) and the dose of [5-^{13}C]glutamine (eqn. 5).

$$R_{a,Gln} = d \cdot (Gln_{inf.}/Gln_{APE}) \quad (5)$$

The rate of leucine C-2 incorporation into glutamine ($R_{a,13}$) was calculated from the product of the glutamine production rate ($R_{a,Gln}$) and the fraction of the leucine flux derived from glutamine (F_{Gln}).

$$R_{a,13} = F_{Gln} \cdot R_{a,Gln} \quad (6)$$

The ratio of leucine C-2 incorporated into glutamine to leucine oxidized ($F_{oxid.}$) is given by eqn. (7). $Leu_{ox.}$ is derived from the [1-^{13}C]leucine data.

$$F_{oxid.} = R_{a,13}/Leu_{ox.} \quad (7)$$

Glutamine oxidation

Glutamine oxidation was calculated from eqn. (8).

$$Gln_{ox.} = (CO_{2,APE} \cdot V_{CO_2})/(Gln_{APE} \cdot 0.9) \quad (8)$$

Where $Gln_{ox.}$ is the glutamine oxidation rate, $CO_{2,APE}$ the exhaled CO_2 enrichment, V_{CO_2} the rate of CO_2 production, Gln_{APE} the plasma glutamine enrichment and 0.9 the correction for metabolic sequestration of CO_2.

Statistics

Statistical significance of the data was determined by two-tailed *t*-tests.

RESULTS

The results are summarized in Tables 1–4. Results in the Tables are means ± S.E.M. Where numerical values are given in the text, the values for the control rats are given first. The endogenous leucine production ($R_{a,21}$, ~ protein breakdown) rate was increased in the septic rats as measured with either [1-^{13}C]leucine (from 246 ± 45 to 363 ± 22 μmol/h per kg; $P < 0.05$; Table 1) or [1,2-^{13}C]leucine (521 ± 80 versus 833 ± 75 μmol/h per kg; Table 2; $P < 0.05$). The leucine oxidation rate was significantly increased with sepsis when the [1-^{13}C]leucine data (from 413 ± 20 to 574 ± 66 μmol/h per kg; $P < 0.05$; Table 1) were used. Whole-body protein synthesis was unchanged, though (Table 1).

Table 2. [1,2-^{13}C]Leucine kinetics

d is the isotope-infusion rate, Leu_{APE}, KIC_{APE} and $Gln_{1,2Leu\text{-}APE}$ are the ^{13}C enrichments of leucine, KIC and glutamine after the infusion of [1,2-^{13}C]leucine. $R_{a,Leu}$ and $R_{a,21}$ are the total and endogenous rates of appearance of leucine in the plasma, F_{Gln} is the fraction of the glutamine flux derived from leucine. ns, not significant.

Parameter	Value		$P <$
	Control	Sepsis	
No. of rats (*n*)	8	8	
Weight of rats (g)	221 ± 5	212 ± 3	ns
d (μmol/h per kg)	147 ± 3	152 ± 5	ns
Infusion vol. (ml/h)	2.12 ± 0.03	2.12 ± 0.07	ns
Leu_{APE} (%)	16.43 ± 1.18	12.29 ± 0.57	0.05
KIC_{APE} (%)	13.27 ± 0.85	11.40 ± 0.51	0.08
$Gln_{1,2Leu\text{-}APE}$ (%)	0.19 ± 0.03	0.32 ± 0.03	0.01
$R_{a,Leu}$ (μmol/h per kg)	792 ± 82	1113 ± 80	0.05
$R_{a,21}$ (μmol/h per kg)	521 ± 80	833 ± 75	0.05
F_{Gln} (%)	1.51 ± 0.31	2.83 ± 0.27	0.01

Table 3. [5-^{13}C]Glutamine kinetics

d is the isotope-infusion rate, Gln_{APE} is the ^{13}C enrichment of plasma glutamine in APE and $R_{a,Gln}$ is the rate of appearance of endogenous glutamine in the plasma. $Gln_{ox.}$ is the rate of glutamine oxidation. ns, not significant.

Parameter	Value		$P <$
	Control	Sepsis	
No. of rats (*n*)	7	9	
Weight of rats (g)	218 ± 4	215 ± 2	ns
d (μmol/h per kg)	64.6 ± 1.4	67.7 ± 1.7	ns
Infusion vol. (ml/h)	2.06 ± 0.03	2.11 ± 0.03	ns
Gln_{APE} (%)	3.22 ± 0.12	2.85 ± 0.11	0.05
$R_{a,Gln}$ (μmol)	1959 ± 94	2331 ± 76	0.01
$Gln_{ox.}$ (μmol)	1089 ± 51	1232 ± 39	0.01

Table 4. Effect of sepsis on the incorporation of leucine C-2 into glutamine

F_{Gln} and F_{Leu} are the fractions of glutamine derived from leucine and of leucine C-2 incorporated into glutamine respectively. $R_{a,13}$ is the rate of leucine C-2 incorporation into glutamine. $F_{oxid.}$ is the ratio of leucine C-2 incorporated into glutamine to leucine oxidized.

Parameter	Value		$P <$
	Control	Sepsis	
F_{Gln} (%)	1.51 ± 0.31	2.83 ± 0.27	0.05
$R_{a,Gln}$ (μmol/h per kg)	1959 ± 94	2331 ± 76	0.01
$R_{a,13}$ (μmol of Leu/h per kg)	29.6 ± 3.7	66.0 ± 6.4	0.01
$Leu_{ox.}$ (μmol of Leu/h per kg)	413 ± 20	574 ± 66	0.05
$F_{oxid.}$ (%)	7.16 ± 0.91	11.49 ± 1.12	0.05

Sepsis caused a 19% increase in the endogenous glutamine production rate ($R_{a,Gln}$; 1959±94 versus 2331±76 μmol/h per kg; $P < 0.05$; Table 3), and, as was found for leucine, the glutamine oxidation rate was increased (1089±51 to 1232±39 μmol/h per kg; $P < 0.05$; Table 3).

There was a significant increase in the rate of leucine C-2 incorporation into glutamine in the septic rats (29.6±3.7 versus 66.0±6.4 μmol/h per kg; $P < 0.01$; Table 4). This increase was due to both an increase in glutamine production ($R_{a,Gln}$, 1959±94 versus 2331±76 μmol/h per kg; $P < 0.01$; Table 3) and an increase in the proportion of glutamine derived from leucine (F_{Gln}; 1.51±0.31% versus 2.83±0.27%; $P < 0.01$; Table 4). The ratio of leucine C_2 incorporated into glutamine to leucine oxidized ($F_{oxid.}$) increased from 7.16±0.91% to 11.49±1.12% ($P < 0.05$) in the septic animals (Table 4).

DISCUSSION

Several rodent models have been developed for studying the response to sepsis *in vivo*. Examples include the use of endotoxin, caecal puncture and injection of live *E. coli*. The advantages of the live *E. coli* model are that it is reproducible, well characterized and does not have the disadvantage of the endotoxin model (questionable relevance to human sepsis) or of caecal puncture (poor reproducibility as well as difficulty in controlling for food intake; Durkot & Wolfe, 1989).

The objectives of this series of experiments were to determine whether oxidation or incorporation into protein were the only pathways for leucine C-2 metabolism in a severe stress state such as sepsis. In stress states leucine carbon atoms could serve as precursors for glutamine in muscle because of the stress-induced insulin resistance decreasing entry of the usual C_2 and C_3 precursors such as glucose from the plasma into the cells. 1,2-^{13}C was used as the tracer, because it was the only commercially available ^{13}C-labelled leucine compound with label in a position other than C-1. Label in the C-1 position is lost during the decarboxylation of isovaleryl-CoA (Scheme 1).

The plasma KIC enrichment was used to calculate both the leucine oxidation rate and the fraction of leucine C-2 transferred to glutamine (F_{Gln}). The rationale for using the KIC enrichment for calculating F_{Gln} is the same as that for using the KIC enrichment to calculate the leucine oxidation rate, namely that KIC is one step nearer to the site of leucine degradation than plasma leucine.

The control values for endogenous leucine production ($R_{a,21}$) are lower than those reported by Vazquez *et al.* (1986) for fed rats (627±5.1 μmol/h per kg). In the present study we found 246±25 μmol/h per kg with [1-^{13}C]leucine (Table 1) and 521±80 μmol/h per kg with [1,2-^{13}C]leucine (Table 2). Long infusions tend to underestimate the leucine flux, owing to recycling (Stein, 1981*a*,*b*). For some unknown reason the values for $R_{a,Leu}$ and $R_{a,21}$ are different for 1-^{13}C and 1,2-^{13}C-labelled leucine (Tables 1 and 2). G.c.–m.s. analysis showed that the both batches of leucine were chemically and isotopically pure. One possible explanation is the presence of a small amount of D-[1-^{13}C]leucine in the [1-^{13}C]leucine. This could result in the accumulation of D-[1-^{13}C]leucine in the plasma and hence in a lower estimate of the leucine flux. A similar problem was reported by McMahon *et al.* (1980) with D-[6,6-$^{2}H_2$]glucose.

Like most other studies of protein metabolism in sepsis we found an increase in the protein breakdown rate as measured by the rate of appearance of endogenous leucine ($R_{a,12}$) using either 1-^{13}C- or 1,2-^{13}C-labelled leucine (Wolfe *et al.*, 1989; Tables 1 and 2). There was no change in the whole-body protein synthesis rate, but the leucine oxidation rate was increased (Table 1).

The values for glutamine production in the control rats ($R_{a,Gln}$; 1959±94 μmol/h per kg; Table 3) are within the range reported in the literature. Lund (1980) reported values for glutamine production ranging from 2820±330 to 1340±80 μmol/h per kg, with the lower values being found with the continuous-infusion technique.

The rates of glutamine appearance in the plasma ($R_{a,Gln}$) and glutamine oxidation ($Gln_{ox.}$) were increased by about 20% with sepsis. The values for glutamine oxidation could have been underestimated, because there may be some dilution of the plasma glutamine at the actual site of glutamine oxidation, but the differences between the two groups are likely to be real.

Glutamine is a major fuel for many rapidly growing cells such as enterocytes, reticulocytes and stimulated lymphocytes. Muscle is the principal site of glutamine synthesis (Newsholme *et al.*, 1988; Rennie *et al.*, 1989). The glutamine is used both as an energy source via glycolysis and to provide precursors for macromolecular synthesis in rapidly growing cells. After injury, lymphocytic and macrophage activity are increased, hence the increased demand for glutamine (Newsholme *et al.*, 1988).

The present study involved isotopic measurements on a secondary pool (glutamine derived from leucine) after the infusion of [1,2-^{13}C]leucine. In rats the primary pool equilibrates with an infused isotopically labelled amino acid in about 3 h (Waterlow *et al.*, 1978). However, a secondary pool, such as [^{13}C]glutamine derived from [1,2-^{13}C]leucine, may take longer to attain an isotopic steady state, especially if the intracellular glutamine pool is large (Elia *et al.*, 1985; Rennie *et al.*, 1989).

To minimize the possibility that the glutamine kinetics were compromised by incomplete equilibration of the secondary pool, we gave a slightly longer (8 h) isotope infusion than is customary for rat studies. Two lines of evidence suggest that 8 h should have been more than adequate. (i) Although the intracellular glutamine concentration is high (~15 mM), both the precursor pool (the tricarboxylic acid cycle) and the end product (plasma glutamine) turn over very quickly. (ii) The situation with [1,2-^{13}C]leucine → intracellular [^{13}C]glutamine → plasma [^{13}C]glutamine is similar to the [^{15}N]glycine → [^{15}N]urea pool → urinary [^{15}N]urea system, where the body urea pool is a secondary pool. Urinary NH_3 reaches a plateau in about 3 h and the urea by about 8 h. If the large urea pool has equilibrated with the infused isotope in 8 h, it is not unreasonable to assume that the comparable glutamine pool had too.

The incorporation of C-2 from leucine into glutamine was increased approximately 2-fold with sepsis (from 29.6±3.7 to 66.0±6.4 μmol/h per kg; Table 4). The increase is significant when compared with the total amount of leucine oxidized (413±20 versus 574±66 μmol/h per kg for the septic rats; Table 1). Thus the fraction of leucine C-2 incorporated into glutamine to leucine C-1 oxidized increased from 7% to 11% (Table 4) with sepsis using the C-1 leucine data (Table 1) for the oxidation rate (eqn. 7).

Muscle is the most likely site of glutamine synthesis from leucine, since muscle is a major site of both leucine oxidation and glutamine synthesis (Askenazi *et al.*, 1980; Newsholme *et al.*, 1988; Rennie *et al.*, 1989). The results suggest that sepsis induces a hypermetabolic state in which tricarboxylic-acid-cycle activity, glutamine synthesis and oxidation are all increased.

The reason for the increased incorporation of leucine C_2 into glutamine is a shift in the tricarboxylic acid cycle away from net oxidation towards anaplerosis, i.e. away from oxidation towards removal of α-oxoglutarate for synthesis of glutamine. The effect is not likely to be specific to leucine; it should apply to all acetyl-CoA entering the tricarboxylic-acid-cycle pool. And, for a net increase in glutamine synthesis to occur, there must also be an influx of other tricarboxylic-acid-cycle intermediates. If at the same time entry of glucose into the tricarboxylic acid cycle is

restricted, there should also be increased utilization of other potential carbon sources, both for acetyl-CoA synthesis and for key intermediates of the tricarboxylic acid cycle.

The results suggest that about 7% of the carbons entering the tricarboxylic acid cycle are used for anaplerosis, and this fraction is increased to ~ 11% with sepsis, a 50% increase. As absolute percentages these values are small, but nevertheless a 50% increase in a rapid process can account for a considerable amount of glutamine synthesis.

In summary, the results show that sepsis induces a hypermetabolic state with increased protein breakdown, leucine oxidation, glutamine synthesis and glutamine oxidation. The contribution of leucine carbon to glutamine synthesis, although small, is increased in septic rats. The most likely site for this to occur is in skeletal muscle. In sepsis, the degradation of leucine C-2 via glutamine is increased because insulin resistance inhibits muscle uptake of other C_3 carbon precursors, such as glucose from the plasma.

This work was supported by National Institutes of Health grants DK 35612 and GM 31828, and by a grant from the Ohtsuka Pharmaceutical Co., Tokushima, Japan.

REFERENCES

Aoki, T. T., Brennan, M. F., Fitzpatrick, G. F. & Knight, D. C. (1981) J. Clin. Invest. **68**, 1522–1528

Askenazi, J., Carpentier, Y. A., Michelson, C. V., Elwyn, D. H., Furst, P., Kantorowitz, L. R., Gump, F. E. & Kinney, J. M. (1980) Ann. Surg. **182**, 78–85

Black, P. R., Brooks, D. C., Bessey, P. Q., Wolfe, R. R. & Wilmore, D. W. (1982) Ann. Surg. **196**, 420–435

Blackburn, G. L., Moldawer, L. L., Usui, S. S., Bothe, A. & O'Keefe, S. J. D. (1979) Surgery **86**, 307–314

Darmaun, D., Manary, M. J. & Matthews, D. E. (1985) Anal. Biochem. **14**, 92–102

Durkot, M. J. & Wolfe, R. R. (1989) J. Surg. Res. **46**, 118–122

Elia, M., Neale, G. & Livesey, G. (1985) Clin. Sci. **69**, 123–133

Hagenfeldt, L., Eriksson, S. & Wahren, J. (1980) Clin. Sci. **59**, 13–18

Lund, P. (1980) FEBS Lett. **117** (suppl.), K86–K92

Lund, P. & Williamson, D. H. (1985) Br. Med. Bull. **41**, 251–256

McMahon, M. M., Schwenk, W. F., Haymond, M. W. & Rizza, R. A. (1980) Diabetes **38**, 97–110

Melville, S., McNurlan, M. A., McHardy, K. C., Broom, J., Milne, E., Calder, A. G. & Garlick, P. J. (1989) Metab. Clin. Exp. **38**, 248–255

Newsholme, E. A., Newsholme, P., Curi, R., Challoner, E. & Ardawi, M. S. M. (1988) Nutrition **4**, 261–268

Odessey, R., Khairallah, E. A. & Goldberg, A. L. (1974) J. Biol. Chem. **249**, 623–629

Rennie, M. J., Maclennan, P. A., Hundal, P. S., Weryk, B., Smith, K., Taylor, P. M., Egan, C. & Watt, P. W. (1989) Metab. Clin. Exp. **38** (suppl. 1), 47–51

Stein, T. P., Leskiw, M. J., Buzby, G. P., Giandomenico, A. L. & Mullen, J. L. (1981*a*) Am. J. Physiol. **239**, E294–E300

Stein, T. P., Buzby, G. P., Leskiw, M. J., Giandomenico, A. L. & Mullen, J. L. (1981*b*) J. Nutr. **111**, 154–167

Stein, T. P., Settle, R. G., Albina, J. A., Melnik, G. & Dempsey, D. T. (1986) J. Nutr. **116**, 1651–1658

Vazquez, J. A., Paul, H. S. & Adibi, S. A. (1986) Am. J. Physiol. **250**, E615–E621

Waterlow, J. C., Garlick, P. J. & Millward, D. J. (1978) Protein Metabolism in Mammalian Tissues and the Whole Body, pp. 339–370, North-Holland Publishing Co., Amsterdam

Wolfe, R. R. (1984) Tracers in Metabolic Research: Radioisotope and Stable Isotope/Mass Spectrometry Methods, pp. 270–271, Alan R. Liss, New York

Wolfe, R. R., Jahoor, F. & Hartl, W. H. (1989) Diabetes/Metabolism Rev. **5**, 149–164

Received 14 March 1990/30 August 1990; accepted 12 November 1990