Infusion of Soy and Casein Protein Meals Affects Interorgan Amino Acid Metabolism and Urea Kinetics Differently in Pigs[1,2,3]

Nicolaas E. P. Deutz,[4] Maaike J. Bruins and Peter B. Soeters

Department of Surgery, Maastricht University, P.O. Box 616, NL-6200 MD Maastricht, The Netherlands

ABSTRACT For routine evaluation of the quality of dietary protein, amino acid scoring patterns were used. Evaluation of this pattern for soy and casein revealed that these proteins are of almost equal quality. However, in vivo studies showed a large difference. To study the biological effects of meals with casein and soy protein, the contributions of individual amino acids to net protein retention and amino acid kinetics in gut, liver and muscle in healthy pigs were investigated. Isonitrogenous enteral nutrition, infused at a rate of 10 mL · kg body $wt^{-1} \cdot h^{-1}$ and consisting of maltodextrin (137 g/L) with added casein (53 g/L) or soy protein (68 g/L), was given to conscious, healthy female multicathetized pigs (20–22 kg, $n = 12$). A primed-constant infusion protocol with L-[ring-2,6-^{3}H]phenylalanine, L-[3,4-^{3}H]valine and [^{15}N-^{15}N]urea was used to measure amino acid and urea kinetics in gut, liver and muscle. Measurements were done postabsorptively and 2–6 h after initiation of the enteral nutrition. During the meal, appearance of amino acids into the portal vein and the uptake by the liver was lower with casein infusion. Muscle uptake did not differ. Gut protein synthesis tended to be lower with soy infusion ($P = 0.1$). Liver protein synthesis and degradation were higher with casein infusion ($P < 0.05$), while in muscle, soy infusion stimulated protein turnover ($P < 0.05$). In comparison to the postabsorptive condition, liver urea production was unchanged after casein infusion, while it was significantly increased after soy infusion. These results suggest that the quality of soy protein is inferior to that of casein protein. J. Nutr. 128: 2435–2445, 1998.

KEY WORDS: • *pigs* • *soy* • *casein* • *interorgan amino acid metabolism* • *isotopes*

The nutritional value of a protein can be estimated by several techniques. Use of the amino acid scoring pattern is that most commonly used because it is reliable and simple, but its validity has been questioned (Fuller and Garlick 1994, Pellett 1990, Sawar 1997). For routine evaluation of the quality of dietary proteins, the FAO/WHO (Pellett 1990) has recommended amino acid scoring patterns corrected for protein digestibility. Whereas biological assays, expressed in numbers for biological value, net protein utilization or protein efficiency rate, have always shown a considerably higher quality of animal proteins, the adoption of the FAO/WHO recommendation in fact leads to an apparent upgrading of the quality of vegetable proteins, compared to animal proteins. The amino acid score is based on estimates of human amino acid requirements, but there is considerable controversy regarding the methods used to determine these requirements. Therefore, the FAO/WHO (Pellett 1990) states that "there is a need for further research to verify and establish human amino acid requirements." The controversy concerning the nutritional value of major dietary proteins like soy and casein in fact is based on the rationale put forward by FAO/WHO that a protein with an amino acid score higher than the reference protein has no additional nutritional value, e.g., the protein digestibility corrected amino acid score for soy protein is calculated to be 0.91. For casein the calculated value is 1.23, yet this value is rounded off to 1.0.

Better, but more laborious techniques, are in vivo techniques, using laboratory animals or humans. These in vivo studies have shown large differences (Sawar 1997, Sawar and Peace 1994) between soy and casein. Important in this respect is the capability of soy to inhibit cell growth, which could have a negative effect on protein turnover (Fotsis 1995, Hawrylewicz et al. 1995, Kennedy 1995, Liener 1995).

The interpretation of in vivo studies dealing with amino acid requirements is subject to debate (Millward 1994, Young and Marchini 1990). Induction of the enzymes of amino acid oxidation when high protein diets are consumed, the amount of dispensable amino acids within the meal, diurnal cycling and many other factors influence estimates of amino acid requirements (Hiramatsu 1994, Millward 1994, Quevedo et al. 1994, Young and Marchini 1990). During feeding only organs with a high protein turnover, like gut and liver, are capable of responding with substantial net protein synthesis. Organs with a low protein turnover, like muscle, consequently will respond only marginally with net protein synthesis on feeding (Waterlow 1995), despite the large mass of muscle. The ability of

[1] This work has been published in abstract form: Deutz N.E.P., Soeters, P. B. Reduced intestinal protein synthesis after a soy protein meal in comparison to a casein protein meal. Clin. Nutr. 1995; 14 Suppl 2: 32. Bruins, M. J., Deutz, N.E.P., Soeters, P. B. Increased liver protein synthesis after a casein meal in comparison to a soy protein meal. Clin Nutr. 1996; 16 Supp 1: 7.

[2] This research was supported by a grant from the European Dairy Association, Brussels, Belgium.

[3] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ''advertisement'' in accordance with 18 USC section 1734 solely to indicate this fact.

[4] To whom correspondence should be addressed.

0022-3166/98 $3.00 © 1998 American Society for Nutritional Sciences.

Manuscript received 22 April 1998. Initial review completed 22 May 1998. Revision accepted 2 September 1998.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

different protein sources to promote net short and long term protein synthesis in different organs and to limit urea production may serve as one of the criteria of protein quality (Quevedo 1994). Our own studies have indicated that net protein synthesis is substantial during a meal and that a large part of this pool is located within the gut (Deutz et al. 1991, 1992 and 1995). In the postabsorptive phase when it is in turn hydrolyzed, protein accumulated in this manner may provide essential amino acids to the body free amino acid pool (Waterlow 1995).

However, the measurement of protein kinetics during a meal is complex. Although during a meal whole body protein synthesis rate is increased (Clugston 1982), interpretation of such data is difficult due to lack of exact knowledge of rates of absorption (Beaufrere et al. 1989). In vivo measurement of amino acid and protein kinetics in important organs like the gut, liver and muscle may circumvent these problems and increase the knowledge regarding amino acid requirements. In addition, this technique will allow estimation of net protein retention in different organs.

When the quality of protein is low, enhanced urea synthesis of the liver is to be expected. A good correlation between plasma urea concentration and protein quality has been reported (Eggum 1976). However, arterial urea concentrations are influenced by several factors like renal function, fluctuations in the amount of total body water and hydration state. Therefore, direct measurement of urea production by the liver is required. The de novo production of urea and the amount of urea recycled can be independently estimated by calculating the rate of appearance of urea after infusion of [^{15}N-^{15}N]urea tracer.

The present study was designed to assess the in vivo quality of casein and soy protein by assessing net protein retention and its kinetics in gut, liver and muscle tissue and the synthesis rate of urea. A pig model was used, because the gastrointestinal tracts of the human and the pig have many similarities. Furthermore, the pig is a good model to study protein quality in vivo because both humans and pigs are highly dependent on the dietary quality of food, due to the minor role of symbiotic microorganisms in modifying ingested nutrients (Miller and Ullrey 1987).

MATERIALS AND METHODS

Animals. Twelve female crossbred [Yorkshire × Dutch Landrace: 20–22 kg body weight (BW)[5]] pigs were housed individually in steel pens (2 × 3 m) with PVC-coated floors, each equipped with an automated watering device. Each morning the pigs were fed a proprietary diet[6] (Zeugenkorrel Universeel Econ, Landbouwbelang, Roermond, The Netherlands), supporting a growth rate of ~300 g per d. The Animal Ethics Committee of the Maastricht University approved the study.

Surgical procedure and postoperative care. The animals had surgery to implant catheters, necessary for measurement of metabolic exchange across portal drained viscera, liver and hindquarter with standardized postoperative care (Deutz et al. 1995 and 1996, Ten Have et al. 1996). In brief, for splanchnic flux measurements, a sample catheter was implanted in the portal vein with its tip in the liver hilus and into an hepatic vein by direct puncture. A catheter for the infusion of para-aminohippuric acid (PAH) was implanted into the splenic vein. For muscle flux measurements, a sample catheter was implanted into the inferior caval vein with its tip 5 cm above the bifurcation, and a PAH infusion catheter was implanted into the abdominal aorta with its tip 5 cm above the bifurcation. For infusion of the tracers, a catheter was implanted into the inferior caval vein with its tip 5 cm above the left renal vein. For infusion of the liquid meal, a catheter was implanted into the stomach. A canvas harness was fitted to each pig to protect the catheters and to allow easy handling of the animal. From the third day onward, the pigs were fed 1 kg of food daily. Throughout the 4 wk period the pigs remained healthy without signs of infection. During sampling the pigs were held in a movable cage (0.9 × 0.5 × 0.3 m), in which they were able to stand and to lie down. The pigs were accustomed to this condition and during the experiment were quietly lying down or asleep.

Four weeks after the operation, the positions of the catheter tips were checked under anesthesia using fluoroscopy. Subsequently, the pig was killed by an intravenous overdose of a barbiturate (Euthanasate, Apharmo, Arnhem, The Netherlands).

Experimental protocol. Two and three weeks after surgery trials were conducted whereby a test diet was given in a crossover design (e.g., half of the pigs received the casein meal at wk 2 and the soy meal at 3 wk and half received the soy meal first and then the casein meal). In the first series of trials in four pigs, only the portal-drained viscera and hindquarter were studied with phenylalanine tracer. In the next eight pigs, also liver metabolism was measured with the phenylalanine, valine and urea tracer. The latter was infused to measure whole body urea kinetics.

The day before a trial (1600 h), food was removed. Normally, food was consumed at that time. On the morning of the trial (0800 h), an infusion of para-aminohippuric acid (PAH, 25 mmol/L, A 1422, Sigma Chemicals, St. Louis, MO) was made through the portal infusion catheter, and the aorta infusion catheter was started at a rate of about 40 mL/h per catheter, after an initial bolus of 5 mL PAH solution (Deutz et al. 1995 and 1996, Ten Have et al. 1996). Infusion of PAH was continued throughout the experimental period. Within 1 h of infusion, steady state plasma PAH concentrations were obtained (not shown).

Directly after the PAH bolus infusion, a primed (137 kBq/kg BW per amino acid) constant and continuous infusion (0.037 MBq · kg $BW^{-1} \cdot h^{-1}$ per amino acid, total volume: 2 mL · kg $BW^{-1} \cdot h^{-1}$) was started of L-[3,4-^{3}H]valine (Amersham, TRK 533, Buckinghamshire, UK) and L-[2,6-^{3}H]phenylalanine (NEN Dupont, NET-493, Mechelen, Belgium) in a 150 mmol/L NaCl solution via the inferior caval vein catheter. Also via this catheter, a primed (135 μmol/kg BW) constant and continuous infusion of 15 μmol · kg $BW^{-1} \cdot h^{-1}$ [^{15}N-^{15}N]urea (99% enrichment in a 150 mmol/L NaCl solution, Cambridge Isotope Laboratories, Andover, MA) was given. In the first four pigs, it was checked that in the period from 45 to 75 min after starting the tracer infusion, tracer steady state was obtained (45 min: 99.6% ± 1.8%, 60 min: 99.3% ± 1.7%, 75 min: 101% ± 2.2%).

One hour after the start of the infusions after taking initial blood samples (control values), constant and continuous infusion of the liquid test meal was started via the gastric catheter. Blood samples were again taken 2 h after the start of the enteral nutrition because pilot experiments (not shown) indicated that at this moment steady state conditions are obtained for the production of substrates by the gut. Also in the first four pigs, it was checked that in the period from 105 to 135 min after start of the enteral nutrition, at ~3 h of tracer infusion, tracer steady state was present (105 min: 98.6% ± 1.9%, 120 min: 101.6% ± 1.6%, 135 min: 100% ± 1.6%). After taking blood samples for the flux measurements, the infusion of the valine and phenylalanine tracers and of PAH was stopped. Infusion of the urea tracer and the liquid enteral meal was continued until 6 h after the start of the liquid enteral meal infusion.

During the experimental period, no other food than the test meals and water was given. Blood was sampled in heparinized cups (Sarstedt, Nümbrecht, Germany) at the intervals indicated.

In a crossover design, an isonitrogenous test meal was infused at a rate of 10 mL · kg $BW^{-1} \cdot h^{-1}$ (for chemical composition, see **Tables 1 and 2**) with either of the following per 100 mL liquid meal: *1)* CAPM (casein protein meal), 5.9 g caseinate and 13.6 g maltodextrin

[5] Abbreviations used: α-AN, α-amino nitrogen; BW, body weight; BCAA, branched chain amino acids; CAPM, casein protein meal; PAH, para-aminohippuric acid; PDV, portal-drained viscera; Ra, rate of appearance; Rd, rate fo disappearance; SOPM, soy protein meal.

[6] Proprietary diet consists of the following (in g/kg food): 149 g crude protein, 55 g crude fat, 55 g ash, 79 g crude cell product, 7.7 g lysine, 9.0 g calcium, 12.9 potassium, 5.3 g phosphorus with added 7500 IE vitamin A, 1500 IE viamin E, 20 mg Cu(II)sulfate, pentahydrate and phytase.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

TABLE 1

Composition of the experimental casein- and soy-based protein meals fed to healthy pigs

	Caseinate	Soy protein
	Units/100 g protein	
Protein, %	89.3 (N × 6.38)	76.9 (N × 6.25)
Nitrogen, %	14.0	12.05
Sodium, *mg*	1402	847
Potassium, *mg*	6	1170
Calcium, *mg*	31	315
Phosphorus, *mg*	797	906
Trypsin inhibitor, *IU*	<730	4550
Isoflavones, *g/g protein*	Not present	0.7

dissolved in demineralized water to 100 mL fluid, or *2)* SOPM (soy protein meal), 6.8 g soy protein and 13.6 g maltodextrin dissolved in demineralized water to 100 mL fluid. All meals were prepared the day before the experiment.

The protein in CAPM was derived from a 1:1:1 mixture of commercially available French, Dutch and Danish sodium caseinate. The protein in SOPM was derived from a 1:1 mixture of soy protein concentrate (Danpro S) and soy protein isolate (Supro 590). The nitrogen and mineral contents in the sodium caseinate and the soy protein are indicated in Table 1.

The test meals contained isonitrogenous amounts (5 g N) of casein or soy protein and equal amounts of maltodextrin (DE20) and were also adjusted with mineral salts to balance the mineral differences in the protein sources. The dry ingredients were dissolved in demineralized water. To ensure complete dissolution, the meals were kept overnight at 4°C until use. As soy proteins typically differ from caseinates with respect to trypsin inhibitor activity and isoflavone content, Table 1 lists these values as well. The trypsin inhibitor activity was measured by standard technique and the isoflavone content estimated from typical values reported in the literature.

Sample processing. During all procedures the samples were put on ice promptly after collection of blood. Haematocrit was determined to enable calculation of plasma flow from blood flow. Centrifugation was performed at 4°C for 5 min at 8900 × *g*. For PAH determinations, 300 μL of whole blood was added to 600 μL of 120 g/L trichloroacetic acid solution, thoroughly mixed and centrifuged, after which the supernatant was collected. To obtain plasma, blood was centrifuged. Plasma (900 μL) was collected for ammonia, urea, the enrichment of urea, glucose and lactate determination, and within 15 min 50 μL 500 g/L trichloroacetic acid solution was added to this plasma, ensuring stability of the substances. Then the solution was mixed, frozen in liquid nitrogen and stored at −80°C until further analysis. For amino acid analysis and measurement of the specific activity of phenylalanine and valine, 500 μL of plasma was deproteinized with 20 mg dry sulfosalicylic acid, mixed, frozen and stored as described above.

Biochemical analysis. Plasma ammonia, urea, glucose and lactate were detected spectrophotometrically by standard enzymatic methods, and PAH was detected spectrophotometrically after deacetylation of the supernatant at 100°C for 45 min (Deutz et al. 1995 and

TABLE 2

Infusion rates of glucose residues and amino acids present in the enteral protein meals with casein or soy protein fed to healthy pigs and measurable by the flux measurements[1]

Substance	CAPM[2]	SOPM[3]
	μmol · kg BW^{-1} · min^{-1}	
Glucose	135.98	135.98
Serine	4.89	4.13
Histidine	1.66	1.37
Glycine	2.20	4.64
Threonine	3.28	2.81
Arginine	1.88	3.58
Alanine	3.07	3.99
Tyrosine	2.79	1.82
Valine	5.03	3.56
Methionine	1.76	0.82
Isoleucine	3.51	3.05
Phenylalanine	2.74	2.67
Leucine	6.42	5.05
Lysine	4.89	3.54
BCAA	14.96	11.66
Sum α-amino gropus	58.50	52.45

[1] The sum of the α-amino groups is from the measurable amino acids in protein. The manufacturers gave the amino acid composition of the meals.
[2] Caseinate and maltodextrin meal.
[3] Soy protein and maltodextrin meal. BCAA, branched chain amino acids.

TABLE 3

Plasma flow in the postabsorptive state (control) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs[1]

Organ	n	Control	CAPM	SOPM
		mL · kg BW^{-1} · min^{-1}		
Portal drained viscera	9	39 ± 2	48 ± 3	46 ± 7
Liver	6	49 ± 5	66 ± 7	60 ± 10
Hindquarter	11	24 ± 2	26 ± 3	28 ± 4

[1] Values are mean ± SEM. No significant differences were observed.

TABLE 4

The ratio between the amount of glucose and amino acid released into the portal vein and their enteral infusion rate after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs[1]

Substrate	CAPM	SOPM
	% of intake	
Glucose	64.5 ± 10.8	65.1 ± 10.6
SER	41.4 ± 7.4	59.8 ± 7.7[b]
HIS	54.8 ± 8.3	80.7 ± 10.5[b]
GLY	41.7 ± 21.9	73.1 ± 12.3
THR	38.7 ± 5.9	71.7 ± 6.9[b]
ARG	57.9 ± 7.3	68.8 ± 15.6
ALA	160.8 ± 20.9	140.2 ± 14.3
TYR	38.7 ± 12.9	61.2 ± 9.8[a]
VAL	53.5 ± 7.5	70.2 ± 10.4[a]
MET	60.7 ± 12.2	52.5 ± 9.4
ILE	56.4 ± 9.8	75.4 ± 9.3[a]
PHE	51.3 ± 7.6	63.9 ± 9.3
TRP	39.5 ± 9.8	61.1 ± 14.0
LEU	42.9 ± 6.7	61.2 ± 10.7[a]
LYS	60.8 ± 9.3	69.5 ± 16.9
BCAA	49.6 ± 7.2	67.7 ± 9.9[a]

[1] Values are mean ± SEM; $n = 10$. [a]$P < 0.05$, [b]$P < 0.01$ versus CAPM group. BCAA, branched chain amino acids.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

TABLE 5

The ratio between the amount of amino acid ingested of phenylalanine and valine and their portal-drained viscera rate of appearance into the portal vein during enteral protein meals with casein or soy protein in healthy pigs[1]

	n	CAPM	SOPM
		% intake	
Phenylalanine	10	80.5 ± 10.4	78.8 ± 9.4
Valine	6	90.2 ± 21.9	100.1 ± 14.2

[1] Values are mean ± SEM. No significant differences were observed.

1996, Ten Have et al. 1996) on an automated analysis system (Cobas Mira-S, Hoffmann-La Roche, Basel, Switzerland).

Plasma amino acids were determined with a fully automated HPLC system after precolumn derivatization with O-phthaldialdehyde (van Eijk 1993). Also, amino acid fractions were collected and counted for radioactivity (van Eijk 1994) to obtain the specific activity of the amino acids. Although phenylalanine can be hydroxylated to tyrosine, we could not detect sufficient radioactivity in the tyrosine fraction to calculate a reliable specific activity of tyrosine.

The enrichment of urea was determined by Gas Chromatography-Mass Spectrometer (Finnigan, Veenedaal, The Netherlands) on the *N,N′*,-bistrimethylsilyl derivative of urea (Wolfe 1992).

Calculations. From all pigs the data obtained in the postabsorptive condition were pooled, representing the control condition.

Plasma flow was calculated using PAH as an indicator in an indicator-dilution calculation (Ten Have et al. 1996). In brief, PAH is infused into the splenic vein and abdominal aorta. The dilution of PAH across the organ depends on the blood flow. Thus, blood flow equals the amount of PAH infused per min divided by the PAH concentration differences in blood downstream (=venous blood) and upstream (=arterial blood) of the organ measured. For PDV flow, venous equals portal venous blood, for liver flow, venous equals hepatic venous and for hindquarter flow, venous equals inferior caval venous blood.

Substrate fluxes were calculated by multiplying the plasma venous − arterial concentration difference by the mean plasma flow (Bloomgarden et al. 1981, Deutz et al. 1992, 1995 and 1996, Rerat et al. 1992, Ten Have et al. 1996). Therefore, a positive flux means production and a negative flux means uptake. Because there was no involvement observed of erythrocytes in intestinal and liver transport of amino acids, plasma fluxes were calculated (Lobley et al. 1996).

Organ valine and phenylalanine turnovers were calculated in a two compartment model (de Blaauw et al. 1996, Wolfe 1992). The tracer balance across an organ (nb), the rate of appearance (Ra) and disappearance (Rd) across the portal-drained viscera (PDV), the splanchnic region and the hindquarter were calculated as follows:

$$nb = (F_{in} \cdot SA_{art}) - (F_{out} \cdot SA_{ven}), (1)$$

$$Rd = nb/SA_{ven}, (2)$$

$$Ra = Rd - (F_{in} - F_{out}). (3)$$

F_{in} is the amount of tracee coming into the organ and is calculated as plasma flow times arterial concentration. F_{out} is the amount of tracee leaving the organ and is calculated as plasma flow times venous concentration. SA_{art} and SA_{ven} are the specific activities of the measured amino acid in the arterial plasma and venous plasma, respectively. Liver Ra and Rd were calculated by subtracting the portal-drained viscera Ra and Rd from the splanchnic Ra and Rd, respectively.

The venous specific activity was used for the calculation of the Rd, because in the postabsorptive state the venous specific enrichment most closely approximates the intracellular specific activity (Biolo et al. 1995). During feeding, all the amino acids coming from the food will dilute the intracellular specific activity and consequently the portal venous specific activity. For this reason it is likely that also during feeding, the venous specific activity most closely reflects the intracellular specific activity.

The whole body urea production (Ra_T), the recycled urea produc-

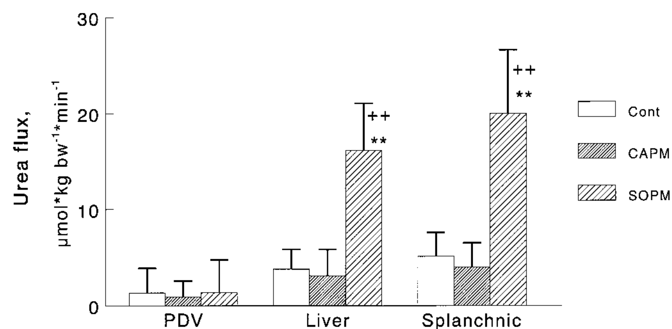

FIGURE 2 Flux of urea across the portal-drained viscera (PDV, $n = 10$), liver ($n = 6$) and splanchnic organs ($n = 7$) in the postabsorptive state (cont) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs. Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$ versus control group, $^{+}P < 0.05$, $^{++}P < 0.01$ SOPM versus CAPM group.

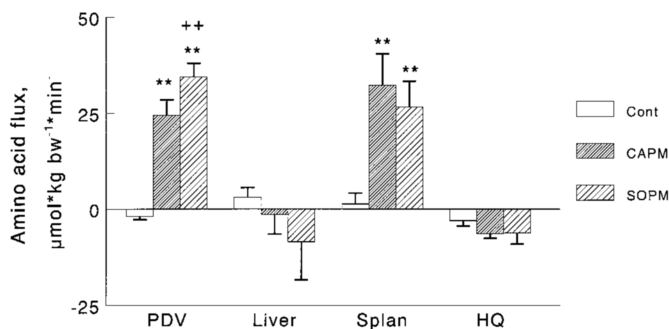

FIGURE 1 Flux of the sum of α-amino acids across the portal-drained viscera (PDV, $n = 10$), liver ($n = 6$), splanchnic organs ($n = 7$) and hindquarter ($n = 11$) in the postabsorptive state (cont) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs. Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$ versus control group, $^{+}P < 0.05$, $^{++}P < 0.01$ SOPM versus CAPM group.

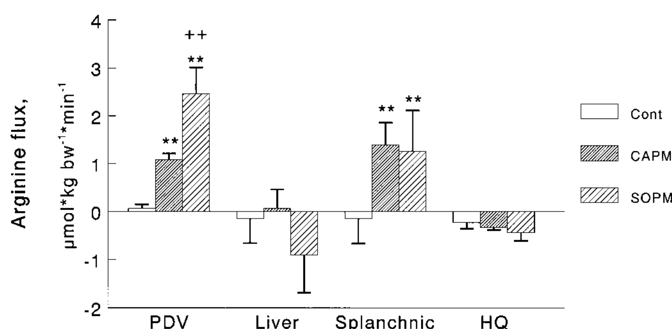

FIGURE 3 Arginine amino acid flux across the portal-drained viscera (PDV, $n = 10$), liver ($n = 6$), splanchnic organs ($n = 7$) and hindquarter ($n = 11$) in the postabsorptive state (cont) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs. Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$ versus control group, $^{+}P < 0.05$, $^{++}P < 0.01$ SOPM versus CAPM group.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

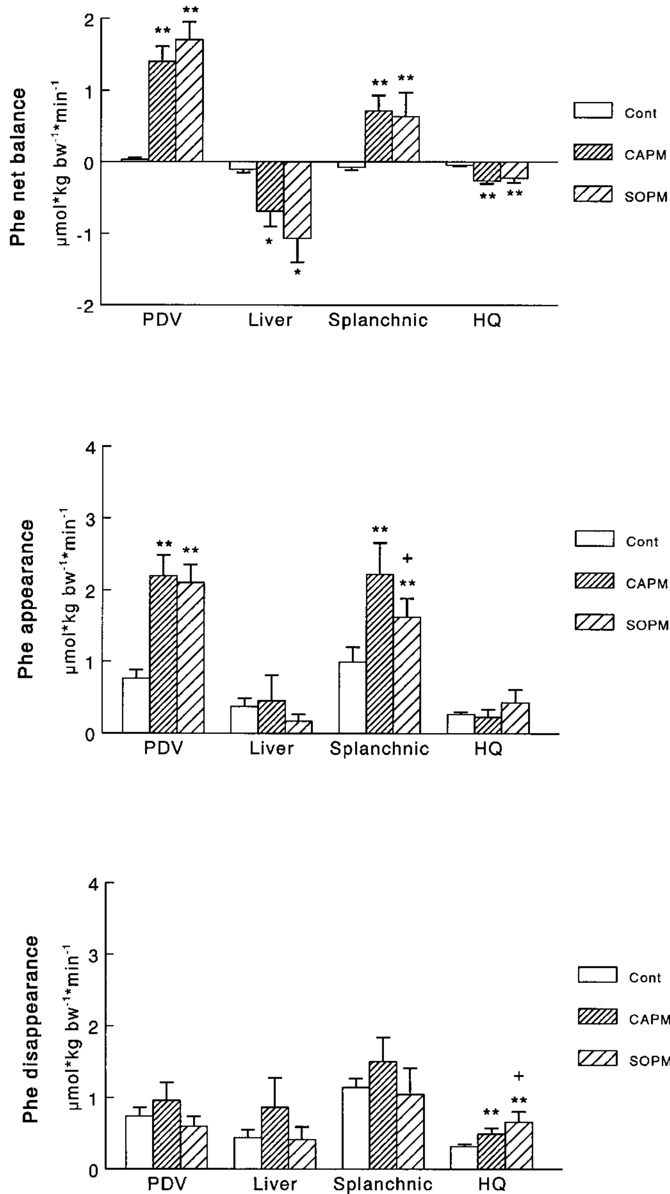

FIGURE 4 Tracee net balance (=amino acid flux), rate of appearance and disappearance of phenylalanine across the portal-drained viscera (PDV, $n = 10$), liver ($n = 6$), splanchnic organs ($n = 7$) and hindquarter ($n = 11$) in the postabsorptive state (cont) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs. Values are mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group, $^{+}P < 0.05$, $^{++}P < 0.01$ SOPM versus CAPM group.

tion (Ra_R) and the de novo urea production (Ra_N) were calculated using the following formulae (Jahoor and Wolfe 1987, Wolfe 1992):

$$Ra_T = I/E^{art}{}_{m+2}, \quad (4)$$

$$Ra_R = I \cdot E^{art}{}_{m+1}/(E^{art}{}_{m+2} \cdot (E^{art}{}_{m+1} + 2 \cdot E^{art}{}_{m+2})), \quad (5)$$

$$Ra_N = Ra_T - Ra_R. \quad (6)$$

I is the amount of tracer infused. $E^{art}{}_{m+1}$ and $E^{art}{}_{m+2}$ are the enrichment of urea in arterial blood with mass + 1 and mass + 2, respectively. The amount of urea, infused into the gut by the enteral nutrition, was [<0.1 μmol · kg BW^{-1} · min^{-1} (not shown) and thus negligible.

During the postabsorptive condition, tracer urea M + 2 enrichment steady state was present, as indicated by the comparable Ra_T value at $t = 0$ and $t = 2$ h. However, for the M + 1 urea enrichment, steady state probably is not present at $t = 0$ h, but only at $t = 6$ h, ~7 h of urea tracer infusion.

α-amino nitrogen (α-AN) was calculated as the sum of the α-amino acids measured. Branched chain amino acids (BCAA) were calculated as the sum of valine, leucine and isoleucine. Because of occlusion of catheters during some of the experiments, data are from fewer animals, as indicated in the legends.

Statistics. Results are presented as means ± SEM. Levels of significance were set at $P < 0.05$. The data were subjected to an ANOVA (SPSS Inc. 1997) with post hoc testing using the Duncan procedure. The data on urea kinetics were subjected to a two-way ANOVA (time and group) (SPSS Inc. 1997) in the interval $t = 0$ h to $t = 6$ h.

RESULTS

Two hours after the initiation of the continuous enteral feeding of CAPM or SOPM, plasma flow of the portal drained viscera, liver and hindquarter did not increase ($P > 0.1$, **Table 3**).

Net flux measurements. The amount of BCAA appearing in the portal vein after 2 h was less than the enteral infusion rate. When calculating the percentage of this amount appearing in the portal vein relative to the BCAA enteral infusion rate (**Table 4**), this percentage was lower for all three BCAA when pigs were infused with CAPM rather than SOPM ($P < 0.05$). Also, this percentage was lower for most other amino acids, including the aromatic amino acids but not significantly for lysine ($P > 0.05$, Table 4). The ratio between the rate of appearance of phenylalanine and valine relative to their enteral infusion rate (**Table 5**) was higher than the ratio relative to the portal drained viscera production (Table 4). This is due to the fact that the rate of appearance is a combination of phenylalanine or valine production by uptake from the enteral lumen and protein breakdown in the gut wall. This ratio was 80% for phenylalanine and did not differ between pigs fed the CAPM and SOPM meals. For valine, this ratio was almost 100% and also did not differ between the two infusions.

The portal drained viscera flux of α-amino nitrogen was higher when SOPM was infused than when CAPM was infused (**Fig. 1**). Because the amount of α-amino acids in the SOPM meal was lower than in the CAPM meal (Table 2), more amino acids must be retained in the portal drained viscera during CAPM feeding. For pigs fed SOPM, the liver uptake and the splanchnic production did not differ ($P > 0.1$). Muscle α-amino acid uptake also did not differ between the pigs fed the two different meals but was small in comparison to splanchnic production.

The liver urea production was ~13 μmol · kg BW^{-1} · min^{-1} greater when pigs were infused with SOPM than with CAPM (**Fig. 2**). Also, no increase relative to the control, postabsorptive values was observed when CAPM was infused. This difference could not be explained by a difference in PDV ammonia production (postabsorptive: 5.7 ± 0.3, CAPM: 9.5 ± 0.6, SOPM: 9.3 ± 0.5 μmol · kg BW^{-1} · min^{-1}) or by the unchanged liver α-amino acid uptake when SOPM was infused (Fig. 1). Therefore, we assume that the main substrates for urea synthesis are not only blood-borne amino acids or ammonia but possibly also are derived from endogenously produced amino acids such as those derived from protein breakdown.

To illustrate the control of amino acid flux by the splanchnic region, arginine fluxes are shown in **Figure 3**. There was a large difference between the amount of arginine infused during the CAPM and SOPM treatments (Table 2). This explains the much higher PDV arginine production during SOPM infusion (Fig. 3). However, the uptake of arginine by the liver

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

TABLE 6

Tracee net balance (NB = amino acid flux) and rate of appearance (Ra) and disappearance (Rd) of phenylalanine across the portal-drained viscera (PDV), liver, splanchnic organs and hindquarter in the postabsorptive state (control) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs[1]

	n	Control	CAPM	SOPM	*P* (ANOVA)
			nmol · kg BW^{-1} · min^{-1}		
PDV	10				
NB		32 ± 29	1403 ± 206[b]	1704 ± 247[b]	0.001
Ra		772 ± 113	2234 ± 269[b]	2300 ± 226[b]	0.001
Rd		740 ± 122	958 ± 256	596 ± 141	NS
Liver	6				
NB		−123 ± 59	−832 ± 157[a]	−914 ± 510[a]	0.001
Ra		361 ± 107	394 ± 354	226 ± 140	NS
Rd		438 ± 112	863 ± 411	414 ± 174	NS
Splanchnic	7				
NB		−72 ± 41	715 ± 215[b]	635 ± 336[b]	0.001
Ra		1071 ± 137	2218 ± 440[b]	1682 ± 263[bc]	0.007
Rd		1143 ± 124	1502 ± 341	1047 ± 364	NS
Hindquarter	11				
NB		−46 ± 13	−263 ± 40[b]	−225 ± 65[b]	0.001
Ra		265 ± 34	226 ± 106	430 ± 176	NS
Rd		312 ± 35	489 ± 81[b]	656 ± 145[bc]	0.005

[1] Values are mean ± SEM. [a]$P < 0.05$, [b]$P < 0.01$ versus control group, [c]$P < 0.05$, [d]$P < 0.01$ versus CAPM group. NS, $P \geq 0.05$.

also was higher in SOPM-infused pigs, resulting in an splanchnic arginine production and hindquarter uptake that did not differ from values during CAPM infusion.

Protein kinetics. The rate of disappearance of phenylalanine, reflecting protein synthesis, in the PDV (**Fig. 4, Table 6**) during the meal was not higher during CAPM infusion than during to the postabsorptive period. However, the rate of disappearance in the SOPM-infused pigs tended to be lower than in the postabsorptive state ($P = 0.1$). Although the net phenylalanine balance in the PDV of the SOPM-infused pigs was slightly higher ($P > 0.1$), the rate of appearance of the PDV did not differ between SOPM and CAPM infusion periods.

The rates of appearance and disappearance of phenylalanine in the liver were not significantly different between SOPM and CAPM infusions.

The rate of disappearance of phenylalanine from muscle (Fig. 4, Table 6) was higher after infusion with SOPM, while the net balance did not differ. Apparently during SOPM infusion, the rate of protein synthesis is stimulated in muscle without net protein synthesis.

To calculate the protein synthesis and protein breakdown rates of the liver, the rates of appearance and disappearance of valine across the liver were measured (**Fig. 5, Table 7**). The rates of appearance and disappearance of valine with CAPM infusion was increased relative to the postabsorptive state and compared to the SOPM infusion, which was also reflected in the splanchnic turnover rates. However, there were no significant differences in net valine balance across the liver.

The whole body rate of appearance (**Table 8**) of phenylalanine was increased by 1.3 μmol · kg BW^{-1} · min^{-1} and did not differ between CAPM and SOPM infusions. The rate of valine appearance increased with 2.1 μmol · kg BW^{-1} · min^{-1} during CAPM infusion and 0.9 μmol · kg BW^{-1} · min^{-1} during SOPM infusion. For both phenylalanine and valine, the increase in the whole body rate of appearance was much less than the amount of amino acid given by the meal.

During the meal, a large increase of the arterial concentration of substrates was observed ($P < 0.001$, **Table 9**).

Urea kinetics. Infusion of [^{15}N-^{15}N]urea enables the measurement of whole body urea Ra_T and Ra_R (**Fig. 6**). In contrast to urea production, measured by direct measurement of the production of urea, Ra_T was not higher at $t = 2$ h. However at $t = 6$ h, Ra_T was higher ($P < 0.001$) during SOPM infusion compared to CAPM infusion. Enteral nutrition increased the amount of urea recycling relative to the whole body Ra_T ($P = 0.003$), but urea recycling did not differ significantly between SOPM and CAPM infusions.

DISCUSSION

This study shows that after a soy protein meal more urea is produced by the liver than after a casein protein meal. This implies that more of the soy protein than the casein protein is degraded. This raises the question of what has happened with the casein protein-derived amino acids that are not converted to urea. The net production by the PDV of essential amino acids was lower during the CAPM infusion, despite a similar absorption. The higher PDV production with SOPM infusion was offset by a higher uptake of nonessential amino acids by the liver, which may have given rise to the higher urea production observed in the liver. In contrast to a significant difference in net production, the components of this net release of amino acids into the portal vein after SOPM did not convincingly reveal whether decreased protein synthesis after SOPM or increased degradation were responsible for increased appearance of amino acids into the portal vein and correspondingly increased urea production by the liver.

Models used to measure protein kinetics. The use of a two-compartimental model (Barrett et al. 1987, Wolfe 1992) enabled the measurement of both the rate of disappearance and appearance of the tracee amino acid. When using phenylalanine tracer, in the gut and muscle the rate of disappearance is related to protein synthesis, because phenylalanine is not oxidized in these tissues (Biolo et al. 1995) and the rate of appearance to protein breakdown. When using valine tracer, the rate of disappearance is largely reflecting protein synthesis and the rate of appearance is reflecting protein breakdown in

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

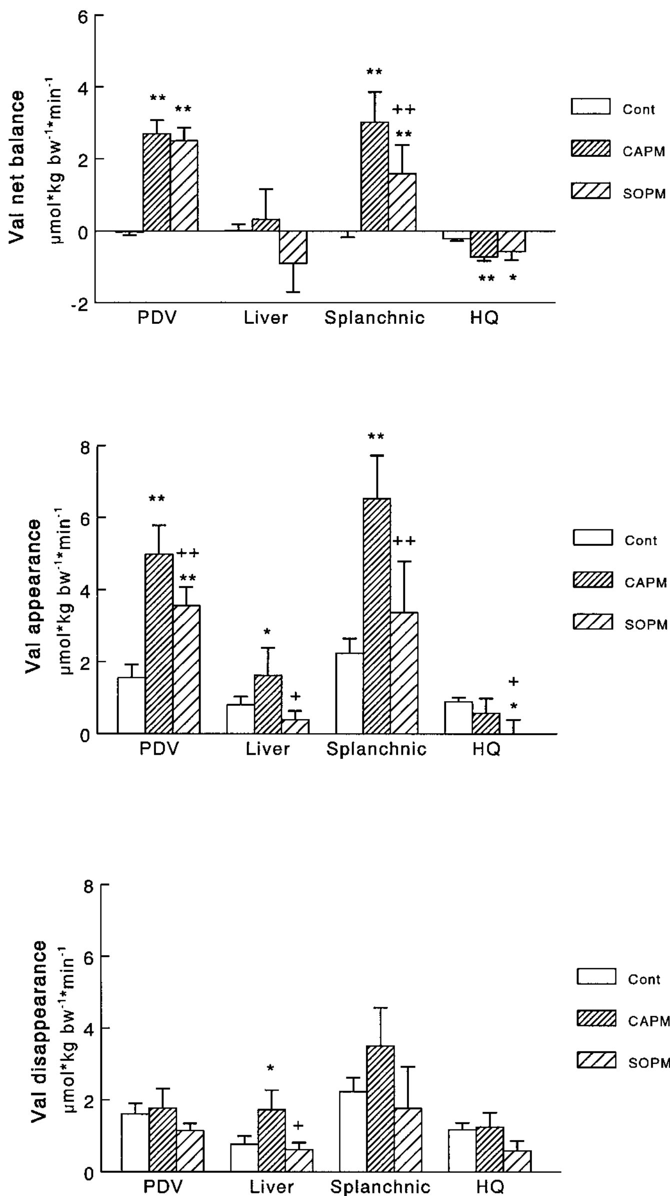

FIGURE 5 Tracee net balance (=valine amino acid flux), rate of appearance and disappearance of valine across the portal-drained viscera (PDV, $n = 6$), liver ($n = 6$), splanchnic organs ($n = 7$) and hindquarter ($n = 5$) in the postabsorptive state (cont) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs. Values are mean ± SEM. *$P < 0.05$, **$P < 0.01$ versus control group, $^{+}P < 0.05$, $^{++}P < 0.01$ SOPM versus CAPM group.

the liver because the transamination rate of valine and subsequently irreversible degradation is very low in the liver (Shinnick and Harper 1976). The disadvantage of the two-compartment model is that it does not take into account recycling of amino acid within the cell (Wolfe 1992).

During enteral nutrition, a large amount of unlabeled amino acids were released into the portal vein. For that reason, calculation of protein breakdown inside the gut wall from the rate of appearance is very difficult. In our model the rate of appearance consisted of unlabeled amino acid coming from protein breakdown within the portal drained viscera tissue and unlabeled amino acid from protein hydrolyzed within the lumen or gut wall and taken up by the gut. During feeding anabolic substrates and hormones from the gut lumen amino acids and from the arterial blood are delivered in the gut. If we assume that during feeding the gut is anabolic, protein degradation must be lower than the rate of disappearance. The rate of appearance should then mainly consist of amino acids derived from enterally delivered proteins, and thus the ratio between the amino acids given and the rate of appearance represents mainly amino acids from the digested protein. This rate of appearance is probably also not influenced by protein released from endogenous sources, like pancreas enzymes, because these are synthetized during feeding (Davis et al. 1996, Simon et al. 1983) and will have approximately the same specific activity as the precursor pool.

Normal response to enteral nutrition. The normal response to enteral nutrition containing casein is an increase in the rate of protein synthesis and oxidation (Clugston and Garlick 1982). Both in the gut and muscle an increase of protein synthesis was observed, measured by the flooding-dose technique (Davis et al. 1996). Data using continuous infusion models are lacking for the gut, but in muscle an increase of protein synthesis was observed (Cheng et al. 1987). In our study, we also observed an increase in muscle protein synthesis. However, we only observed a tendency for the rate of protein synthesis to increase in the gut. This possibly is due to the higher variance in our AV-model in comparison to the flooding dose technique.

In the case of anabolism of the gut during feeding, the net uptake of amino acids by gut tissue for protein synthesis during enteral nutrition must be less than the rate of disappearance (CAPM: 1 μmol · kg BW^{-1} · min^{-1}, Fig. 4, *bottom*, Table 8). The amount of phenylalanine ingested was 2.7 μmol · kg BW^{-1} · min^{-1} (Table 2), and the net balance of phenylalanine was 1.5 μmol · kg BW^{-1} · min^{-1} (Fig. 4, Table 8). If we assume that the uptake from the hydrolyzed casein in the lumen of phenylalanine was ~80% (Table 4), then the net uptake of phenylalanine, coming from the hydrolyzed casein protein, by the gut was ~0.7 μmol · kg BW^{-1} · min^{-1} (0.8 · 2.7–1.5), ~25% of the enteral intake. In the case of a complete enteral uptake, this will amount to ~45% of the intake. Also, because protein synthesis does not increase compared to the postabsorptive condition, it can be concluded that during nutrition, decreased protein breakdown and unchanged protein synthesis is the cause of increased protein accretion in the gut. At the whole body level, we found an increase in phenylalanine appearance of 1.3 μmol · kg BW^{-1} · min^{-1}, suggesting reduced protein breakdown, which is consistent with other observations (Biolo et al. 1992, Motil et al. 1981).

In the liver, protein synthesis and degradation were increased after infusion of the casein meal. The response of increased liver protein synthesis during and after enteral nutrition is in agreement with a study in pigs (Davis et al. 1996). However, a significant net uptake of valine was not observed. This may mean that for the net synthesis of protein in the liver valine from intracellular sources, probably from protein breakdown, was used. During enteral nutrition, an increase in the synthesis of albumin in the liver was observed (Cayol et al. 1996, de Feo et al. 1992) and therefore the observed protein synthesis may be due to an increased synthesis of albumin and possibly of other plasma proteins. The rate of appearance of phenylalanine is an indication of protein breakdown. The increased rate of disappearance of phenylalanine is a combination of protein synthesis and hydroxylation of phenylalanine to tyrosine. Therefore, a change in the ratio between the rate of disappearance of valine and the rate of disappearance of phenylalanine is an indication of a change in the percentage of hydroxylation of phenylalanine. However, this was not

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

TABLE 7

Tracee net balance (NB = amino acid flux) and rate of appearance (Ra) and disappearance (Rd) of valine across the portal-drained viscera (PDV), liver, splanchnic organs and hindquarter in the postabsorptive state (control) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs[1]

	n	Control	CAPM	SOPM	*P* (ANOVA)
			nmol · kg BW^{-1} · min^{-1}		
PDV	6				
NB		−28 ± 85	2692 ± 378[b]	2497 ± 369[b]	0.001
Ra		1609 ± 336	4976 ± 801[b]	3558 ± 507[bd]	0.001
Rd		1610 ± 294	1774 ± 549	1153 ± 196	NS
Liver	6				
NB		19 ± 170	325 ± 836	904 ± 792	NS
Ra		805 ± 228	1619 ± 764[a]	390 ± 242[c]	0.05
Rd		770 ± 222	1733 ± 549[a]	622 ± 196[c]	0.05
Splanchnic	7				
NB		−3 ± 169	3018 ± 836[b]	1592 ± 792[bd]	0.001
Ra		2234 ± 417	6525 ± 1209[b]	3368 ± 1414[d]	0.04
Rd		2237 ± 387	3507 ± 1064	1775 ± 1157	NS
Hindquarter	5				
NB		−217 ± 59	−724 ± 115[b]	−571 ± 235[a]	0.004
Ra		892 ± 113	571 ± 413	4 ± 387[ac]	0.04
Rd		1181 ± 185	1254 ± 399	596 ± 269	NS

[1] Values are mean ± SEM. [a]$P < 0.05$, [b]$P < 0.01$ versus control group, [c]$P < 0.05$, [d]$P < 0.01$ versus CAPM group.

observed. Phenylalanine turnover is lower in SOPM-infused pigs, which also is consistent line with the decrease in the turnover of valine that was observed.

When calculating the amount of valine escaping the splanchnic region in comparison to the amount of valine taken up from the gut lumen and released by the gut into the portal vein (rate of appearance) (Fig. 5, Table 7), the splanchnic region utilizes ~50% of the intake for its own protein synthesis. For phenylalanine, this amounted to 70%. The splanchnic region indeed is a very important site of protein anabolism during enteral feeding in our model, probably in the order of 50% of total intake. Also, when calculating the relation between the rate of appearance of valine across the splanchnic region (5.9 μmol · kg BW^{-1} · min^{-1}) and the whole body rate of appearance (5.9 μmol · kg BW^{-1} · min^{-1}), it is clear that the production of amino acids by the splanchnic region into the greater circulation determines the amount of protein synthesis within the extra-splanchnic region. In addition, the contribution of the rate of appearance from extra-splanchnic tissue must therefore be very small and indicate reduced protein breakdown in these organs during enteral feeding.

Observations concerning the biological value of casein and soy protein. The differences between the biological effects upon gut and liver of casein and soy protein are evident. Soy protein has a protein turnover reducing effect on gut and liver. This observation is in agreement with the study by Nielsen et al. (1994), in which it was shown that at the whole body level soy protein did not increase protein synthesis and reduced protein breakdown. Casein was found to stimulate protein turnover. During soy feeding, gut metabolism is less stimulated than during casein feeding. Our hypothesis is that stimulation of gut metabolism during feeding has a positive effect on gut function, but this has yet to be proven.

After uptake of protein as the individual amino acids in the gut and subsequent gut metabolism of these amino acids, these amino acids are delivered to the liver. The fourfold increase of net liver urea synthesis above the value during the postabsorptive state after soy feeding implies that the biological value of soy protein is much less than that of casein. When looking at the whole body urea production, measured by the Urea isotope, SOPM also induces more urea production that CAPM. However, the uptake of amino acids and ammonia by the liver from blood is quantitatively not in relation to this urea syn-

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

TABLE 8

Whole body rate of appearance of phenylalanine and valine in the postabsorptive state (control) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs[1]

Substrate	*n*	ANOVA	Control	CAPM	SOPM
				nmol · kg BW^{-1} · min^{-1}	
PHE	12	$P = 0.001$	1708 ± 91	2987 ± 148[b]	2842 ± 181[b]
VAL	7	$P = 0.001$	3821 ± 237	5938 ± 313[b]	4688 ± 383[ad]

[1] Values are mean ± SEM. [a]$P < 0.05$, [b]$P < 0.01$ versus control group, [d]$P < 0.01$ versus CAPM group.

TABLE 9

Arterial concentrations in the postabsorptive state (control) and 2 h after the start of enteral protein meals with casein (CAPM) or soy protein (SOPM) in healthy pigs[1]

Substrate	Control	CAPM	SOPM	*P* (ANOVA)
		μmol/L		
Ammonia	54 ± 2	73 ± 4[b]	76 ± 7[b]	0.001
Urea	3474 ± 386	2730 ± 300	3760 ± 606	NS
Glucose	4927 ± 244	9237 ± 608[b]	7537 ± 521[bd]	0.001
Lactate	801 ± 80	1140 ± 117[a]	983 ± 131	0.05
GLU	230 ± 14	296 ± 26[a]	275 ± 29	NS
ASN	36 ± 3	69 ± 6[b]	109 ± 9[bd]	0.001
SER	117 ± 6	154 ± 8[b]	162 ± 10[b]	0.001
GLN	436 ± 24	478 ± 37	485 ± 20	NS
HIS	45 ± 3	92 ± 6[b]	78 ± 6[bc]	0.001
GLY	766 ± 41	638 ± 44	786 ± 58	NS
THR	92 ± 8	155 ± 13[b]	142 ± 19[b]	0.001
CIT	69 ± 3	88 ± 5[a]	94 ± 9[b]	0.001
ARG	91 ± 6	117 ± 7	206 ± 20[bd]	0.001
ALA	407 ± 52	489 ± 39	551 ± 41	NS
TAU	43 ± 5	42 ± 6	35 ± 5	NS
TYR	35 ± 2	116 ± 11[b]	80 ± 6[bd]	0.001
VAL	246 ± 9	396 ± 24[b]	341 ± 25[bc]	0.001
MET	26 ± 1	59 ± 6[b]	27 ± 1[d]	0.001
ILE	119 ± 7	197 ± 14[b]	198 ± 15[b]	0.001
PHE	51 ± 3	105 ± 6[b]	113 ± 6[b]	0.001
TRP	17 ± 1	32 ± 2[b]	33 ± 4[b]	0.001
LEU	112 ± 5	253 ± 21[b]	211 ± 22[bd]	0.001
ORN	68 ± 9	94 ± 10	123 ± 22[b]	0.006
LYS	81 ± 7	267 ± 18[b]	192 ± 17[bd]	0.001
BCAA	478 ± 19	846 ± 56[b]	751 ± 59[b]	0.001
α-AN	3042 ± 113	4160 ± 192[b]	4253 ± 222[b]	0.001

[1] Values are mean ± SEM, $n = 12$. [a]$P < 0.05$, [b]$P < 0.01$ versus control group, [c]$P < 0.05$, [d]$P < 0.01$ versus CAPM group.

thesis. If we assume that for 1 mol urea 2 mol nitrogen is necessary, there is a deficit in nitrogen uptake from blood. Therefore by exclusion, we may conclude that soy appears to stimulate the breakdown of endogenous protein within the liver. When enteral nutrition is discontinued, this deficit has to be restored and amino acid sources from other organs (e.g., muscle) possibly are used for that purpose. Also, the fact that we did not observe an expected increase in liver protein turnover after soy feeding in comparison to casein is consistent with disturbed liver protein kinetics during soy feeding.

The net uptake of amino acids by muscle is comparable between the casein and soy meals. For the sum of the amino acids, production by the splanchnic region is much larger than uptake by muscle, assuming that hindquarter muscle represents ~50% of total body muscle. However for phenylalanine, an amino acid only used for protein turnover in extra-splanchnic tissues (except the kidney), net splanchnic production is comparable to net uptake by muscle, regardless of the meal given. Therefore, for the other amino acids, nonsplanchnic non-muscle metabolism consumption also is important.

During infusion of the two types of meals, stimulated branched-chain amino acid transamination does not seem to occur, because the ratio between the rate of appearance of phenylalanine and valine is not different (0.7). All the amino acids taken up from the circulation apparently are used for protein synthesis.

Arterial concentrations (Table 9) after enteral nutrition are different between the two types of meals. However, for some amino acids like glycine the change of the arterial concentration is less pronounced than the differences in composition (Table 2), in agreement with a previous study (Hagemeister et al. 1990). For most of the essential amino acids, however, the change in arterial concentration is proportional to the content in protein (e.g., BCAA). We observed that adding carbohydrates to a pure protein meal enhances net protein synthesis in the gut (Deutz et al. 1995). This increase was found to be related to the postmeal insulin response. In the present study, the difference in the arterial concentrations of glucose and amino acids probably

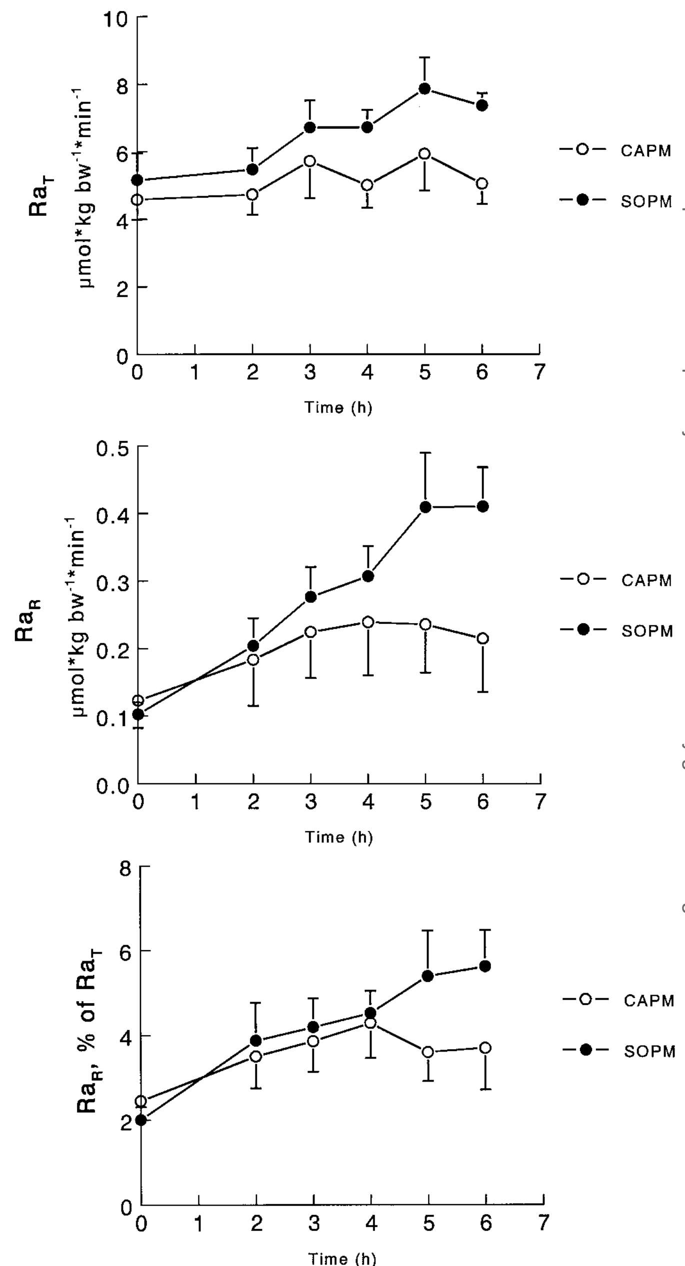

FIGURE 6 Ra_T, Ra_R and Ra_N of urea in the postabsorptive state ($t = 0$ h) and during infusion ($t > 0$ h) of the enteral protein meals with casein or soy protein in healthy pigs. Values are mean ± SEM ($n = 7$). ANOVA, group effect: Ra_T, $P < 0.001$; Ra_R, $P = 0.015$; time effect: Ra_T, $P = 0.049$; Ra_R, $P = 0.003$; Ra_N, $P = 0.011$. No significant interaction was observed.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

also play a role in the anticipated changes in plasma insulin levels and protein kinetics.

Comparison between liver urea production and urea kinetics. We observed a discrepancy between liver urea flux and the Ra_T obtained from the stable isotope dilution. If we calculate the total production of urea during the 6 h of infusion of the meals, SOPM meals induced a production of ~2270 μmol urea and CAPM meals ~1750 μmol urea (cumulative Ra_T) per kg BW. The isonitrogenous meals consisted of 5 g nitrogen per 600 mL nutrition and thus per kg BW were infused with 0.5 g nitrogen or ~71,000 μmol urea (contains 2 N molecules) per 6 h. Thus the production measured with the urea isotope is only a small part of the total amount of nitrogen delivered by the meal. Also, net liver production of urea by the liver during the 6 h, assuming for SOPM that the production observed at $t = 2$ h remains until $t = 6$ h, was during the SOPM infuaion ~6900 μmol and during the CAPM infusion ~1500 μmol per kg BW, only a small part of the total amount of nitrogen delivered by the test meal. This implies that 6 h after a balanced meal most of the amino acids ingested are retained in some form (protein or amino acids) but to a larger extent after the casein meal than after the soy meal.

This study shows that soy protein does not stimulate protein synthesis as much as casein protein, and that during infusion of the meal a proportionally much larger part of the soy protein is degraded to urea than casein protein. This implies that the biological value of soy protein is inferior to that of casein protein. This technique of measuring the biological effects of different types of protein in enteral meals appears to give more information regarding their in vivo effects than other techniques.

ACKNOWLEDGMENTS

The authors express many thanks to H.M.H. van Eijk and D. R. Rooyakkers for analytical help on amino acid analysis and specific activity measurements, A.J.M. Wagenmakers and A. Gijsen for analytical help on urea enrichment measurements, G.A.M. Ten Have, J. Suyk and M.C.F. Bost for help in the operative procedure and G.A.M. Ten Have for help during the experiments. J. Steyns is acknowledged for help in the materials section.

LITERATURE CITED

Barrett, E. J., Revkin, J. H., Young, L. H., Zaret, B. L., Jacob, R. & Gelfand, R. A. (1987) An isotopic method for measurement of muscle protein synthesis and degradation in vivo. Biochem. J. 245: 223–228.

Beaufrere, B., Horber, F. F., Schwenk, F., Marsh, H. M., Matthews, D., Gerich, J. E. & Haymond, M. W. (1989) Glucocorticosteroids increase leucine oxidation and impair leucine balance in humans. Am. J. Physiol. 257: E712–E721.

Biolo, G., Tessari, P., Inchiostro, S., Bruttomesso, D., Fongher, C., Sabadin, L., Fratton, M. G., Valerio, A. & Tiengo, A. (1992) Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach. Am. J. Physiol. 262: E455–E463.

Biolo, G., Zhang, X-J. & Wolfe, R. R. (1995) Role of membrane transport in interorgan amino acid flow between muscle and small intestine. Metabolism 44: 719–724.

Bloomgarden, Z. T., Liljenquist, J., Lacy, W. & Rabin, D. (1981) Amino acid disposition by liver and gastrointestinal tract after protein and glucose ingestion. Am. J. Physiol. 241: E90–E99.

Cayol, M., Boirie, Y., Prugnaud, J., Gachon, P., Beaufrere, B. & Obled, C. (1996) Precursor pool for hepatic protein synthesis in humans: effects of tracer route infusion and dietary proteins. Am. J. Physiol. 270: E980–E987.

Cheng, K. N., Pacy, P. J., Dworzak, F., Ford, G. C. & Halliday, D. (1987) Influence of fasting on leucine and muscle protein metabolism across the human forearm determined using L-[1–13C,15N] leucine as the tracer. Clin. Sci. 73: 241–246.

Clugston, G. A. & Garlick, P. J. (1982) The response of protein and energy metabolism to food intake in lean and obese man. Human Nutr. Clin. Nutr. 36C: 57–70.

Davis, T. A., Burrin, D. G., Fiorotto, M. L. & Nguyen, H. V. (1996) Protein synthesis in skeletal muscle and jejunum is more responsive to feeding in 7- than in 26-day-old pigs. Am. J. Physiol. 270: E802–E809.

de Blaauw, I., Deutz, N.E.P. & von Meyenfeldt, M. F. (1996) In vivo amino acid metabolism of gut and liver during short and prolonged starvation. Am. J. Physiol. 270: G298–G306.

De Feo, P., Horber, F. F. & Haymond, M. W. (1992) Meal stimulation of albumin synthesis: a significant contributor to whole body protein synthesis in humans. Am. J. Physiol. 263: E794–E799.

Deutz, N. E. P., Reijven, P. L. M., Athanasas, G. & Soeters, P. B. (1992) Post-operative changes in hepatic, intestinal, splenic and muscle fluxes of amino acids and ammonia in pigs. Clin. Sci. 83: 607–614.

Deutz, N. E. P., Reijven, P. L. M., Bost, M. C. F., van Berlo, C.L.H. & Soeters, P. B. (1991) Modification of the effects of blood on amino acid metabolism by intravenous isoleucine. Gastroenterology 101: 1613–1620.

Deutz, N.E.P., Ten Have, G.A.M. & Soeters, P. B. (1992) Addition of tryptophan to a gelatin protein meal enhances intestinal retention of meal-derived amino acids. Clin. Nutr. 11(Suppl. 1): 61.

Deutz, N.E.P., Ten Have, G.A.M., Soeters, P. B. & Moughan, P. J. (1995) Increased intestinal amino-acid retention from addition of carbohydrates to a meal. Clin. Nutr. 14: 354–364.

Deutz, N.E.P., Welters, C.F.M. & Soeters, P. B. (1996) Intragastric bolus feeding of meals containing elementary, partially hydrolysed or intact protein causes comparable changes in interorgan metabolism in a pig model. Clin. Nutr. 15: 119–128.

Eggum, B. O. (1976) Indirect measures of protein adequacy. In: Protein metabolism and Nutrition (Cole, D.J.A., Boorman, K. N., Buttery, P. J., Lewis, D., Neale, R. J. & Swan, H., Eds.), pp. 249–258. Butterworths, London.

Fotsis, T., Pepper, M., Adlercreutz, H., Hase, T., Montesano, R. & Schweigerer, L. (1995) Genistein, a dietary ingested isoflavonoid, inhibits cell proloferation and in vitro angiogenesis. J. Nutr. 125: 790S–797S.

Fuller, M. F. & Garlick, P. J. (1994) Human amino acid requirements: can the controversy be resolved? Annu. Rev. Nutr. 14: 217–241.

Hagemeister, H., Scholz-Ahrens, K. E., Schulte-Coerne, H. & Barth, C. A. (1990) Plasma amino acids and cholesterol following consumption of dietary casein or soy protein in minipigs. J. Nutr. 120: 1305–1311.

Hawrylewicz, E. J., Zapata, J. J. & Blair, W. H. (1995) . Soy and experimental cancer: animal studies. J. Nutr. 125: 698S–708S.

Hiramatsu, T., Cortiella, J., Marchini, J. S., Chapman, T. E. & Young, V. R. (1994) . Source and amount of dietary nonspecific nitrogen in relation to whole-body leucine, phenylalanine, and tyrosine kinetics in young men. Am. J. Clin. Nutr. 59: 1347–1355.

SPSS Inc. (1997) SPSS for Windows Version 7.5. SPSS Inc., Chicago, IL.

Jahoor, F. & Wolfe, R. R. (1987) Reassessment of primed constant-infusion tracer method to measure urea kinetics. Am. J. Physiol. 252: E557–E564.

Kennedy, A. R. (1995) The evidence for soybean products as cancer preventive agents. J. Nutr. 125: 733S–743S.

Liener, I. E. (1995) Possible adverse effects of soybean anticarcinogens. J. Nutr. 125: 744S–750S.

Lobley, G. E., Connell, A., Revell, D. K., Bequette, B. J., Brown, D. S. & Calder, A. G. (1996) Splanchnic-bed transfers of amino acids in sheep blood and plasma, as monitored through use of a multiple U-13C-labelled amino acid mixture. Br. J. Nutr. 75: 217–235.

Miller, E. R. & Ullrey, D. E. (1987) The pig as a model for human nutrition. Annu. Rev. Nutr. 7: 361–382.

Millward, D. J. (1994) Can we define indispensable amino acid requirements and assess protein quality in adults? J. Nutr. 124: 1509S–1516S.

Motil, K. J., Matthews, D. E., Bier, D. M., Burke, J. F., Munro, H. N. & Young, V. R. (1981) Whole-body leucine and lysine metabolism response to dietary protein intake in young men. Am. J. Physiol. 240: E712–E721.

Nielsen, K., Kondrup, J., Elsner, P., Juul, A. & Jensen, E. S. (1994) Casein and soya-bean protein have different effects on whole body protein turnover at the same nitrogen balance. Br. J. Nutr. 72: 69–81.

Pellett, P., Bressani, R., Devadas, R., Dillon, J.-C., Eggum, B., Hussein, M. A., Miller, E. L., Oyeleke, O. A., Slump, P., Yoshida, A. & Young, V. R. (1990) Joint FAO/WHO expert consulation on protein quality evaluation. FAO, Bethesda, MD and WHO, Geneva.

Pellett, P. L. (1990) Protein energy requirements in humans. Am. J. Clin. Nutr. 51: 723–737.

Quevedo, M. R., Price, G. M., Halliday, D., Pacy, P. J. & Millward, D. J. (1994) Nitrogen homoeostasis in man: diurnal changes in nitrogen excretion, leucine oxidation and whole body leucine kinetics during a reduction from a high to a moderate protein intake. Clin. Sci. 86: 185–193.

Rérat, A., Simoes-Nunes, C., Mendy, F., Vaissade, P. & Vaugelade, P. (1992) Splanchnic fluxes of amino acids after duodenal infusion of carbohydrate solutions containing free amino acids or oligopeptides in the non-anaesthetized pig. Br. J. Nutr. 68: 111–138.

Sarwar, G. (1997) The protein digestibility-corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. J. Nutr. 127: 758–764.

Sarwar, G. & Peace, R. W. (1994) The protein quality of some enteral products is inferior to that of casein as assessed by rat growth methods and digestibility-corrected amino acid scores. J. Nutr. 124: 2223–2232.

Shinnick, F. L. & Harper, A. E. (1976) Branched-chain amino acid oxidation by isolated rat tissue preperations. Biochim. Biophys. Acta 437: 477–486.

Simon, O., Zebrowska, T., Bergner, H. & Munchmeyer, R. (1983) Investigations on the pancreatic and stomach secretion in pigs by means of continuous infusion of 14C-amino acids. Arch. Tierernahr. 33: 9–22.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022

Ten Have, G.A.M., Bost, M.C.F., Suyk-Wierts, J.C.A.W., van den Bogaard, A.E.J.M. & Deutz, N.E.P. (1996) Simultaneous measurement of metabolic flux in portally-drained viscera, liver, spleen, kidney and hindquarter in the conscious pig. Lab. Anim. 30: 347–358.
van Eijk, H.M.H., Huick, M.P.L., Rooyakkers, D. R. & Deutz, N.E.P. (1994) Automated simultaneous isolation and quantitation of labeled amino acid fractions from plasma and tissue by ion-exchange chromatography. J. Chromatogr. 660: 251–257.
van Eijk, H.M.H., Rooyakkers, D. R. & Deutz, N.E.P. (1993) Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2–3 um Spherisorb ODS II column. J. Chromatogr. 620: 143–148.
Waterlow, J. C. (1995) Whole-body protein turnover in humans- past, present, and future. Annu. Rev. Nutr. 15: 57–92.
Wolfe, R. R. (1992) Radioactive and stable isotope tracers in biomedicine. Principles and practice of kinetic analysis. Wiley-Liss, New York.
Young, V. R. & Marchini, J. S. (1990) Mechanisms and nutritional significance of metabolic responses to altered intakes of protein and amino acids, with reference to nutritional adaptation in humans. Am. J. Clin. Nutr. 51: 270–289.

Downloaded from https://academic.oup.com/jn/article/128/12/2435/4724280 by guest on 20 August 2022