Casein and soya-bean protein have different effects on whole body protein turnover at the same nitrogen balance

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The present study examined whether different proteins have different effects on whole-body protein turnover in adult rats. The rats were either starved, given a protein-free but energy-sufficient diet (1 MJ/kg body weight (BW) per d) or a diet containing intact casein, hydrolysed casein, or hydrolysed soya-bean protein at a level of 9·1 g/kg BW per d. The diets, which were isoenergetic with the same carbohydrate:fat ratio, were given as a continuous intragastric infusion for at least 4 d. During the last 19 h ^{15}N-glycine (a primed continuous infusion) was given intragastrically and ^{15}N was recovered from urinary ammonia and urea during isotope steady state for measurement of protein synthesis and protein degradation. Compared with starvation the protein-free diet decreased N excretion by 75%, probably by increasing the rate of reutilization of amino acids from endogenous proteins for protein synthesis. The protein diets produced a positive N balance which was independent of the protein source. Intact and hydrolysed casein increased protein synthesis 2·6- and 2·0-fold respectively, compared with the protein-free diet. Protein degradation increased 1·4- and 1·2-fold respectively. Hydrolysed soya-bean protein did not increase protein synthesis but decreased protein degradation by 35% compared with the protein-free diet. Compared with the hydrolysed soya-bean protein, intact casein resulted in 2·2- and 2·8-fold higher rates of protein synthesis and degradation respectively. These results are not easily explained by known sources of misinterpretation associated with the ^{15}N-glycine method. Hydrolysed casein and hydrolysed soya-bean protein produced similar concentrations of insulin-like growth factor-1, insulin, glucagon, and corticosterone. The difference in amino acid composition between the dietary proteins was reflected in plasma amino acid composition and this is suggested to be responsible for the different effect on protein turnover. Preliminary results from this study have previously been published in abstract form (Nielsen *et al.* 1991).

Protein turnover: ^{15}N-glycine: Casein: Soya-bean protein: Rat

Different preparations of identical proteins result in different growth rates of rats. Refeeding of starved rats with hydrolysed whey resulted in faster weight gain than refeeding with intact whey (Poullain *et al.* 1989, 1991) and hydrolysed casein gave a higher growth rate than intact casein (Zaloga *et al.* 1991). In the present study it was examined whether different proteins with equal nutritional quality affect whole-body protein synthesis and degradation differently. Adult rats were used in order to measure protein turnover in a state of N balance which reflects protein metabolism rather than regulation of growth. Continuous intragastric infusion of a liquid diet and of ^{15}N-glycine for measurement of whole-body protein turnover was employed.

In the first experiment three different commercial liquid formulas containing intact casein, hydrolysed casein or hydrolysed soya-bean protein were compared with a protein-

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free diet, and with starvation. The three protein sources produced almost the same N balance but intact and hydrolysed casein increased protein synthesis and degradation while hydrolysed soya-bean protein decreased degradation.

In a second experiment the time course of isotope enrichment showed a clear difference in flux only 4–6 h after beginning isotope infusion. A third experiment with specially prepared diets which only differed in the protein source produced the same differences. Plasma concentrations of insulin, insulin-like growth factor 1 (IGF-1), glucagon, corticosterone and individual amino acids were also measured, allowing the tentative explanation that the difference was due to the different amino acid compositions of the proteins.

METHODS

Operative procedures

Female Wistar rats with a body weight of about 250 g (Møllegaard Breeding Center, Denmark) were anaesthetized by two consecutive injections (Hypnorm, Janssen, 0·2 ml/100 g body weight (BW) i.p.; diazepam 0·8 mg/100 g BW i.p.). The hair over the scapula and abdomen was shaved and the skin painted with iodine. Liquid dressing (Nobecutane; Astra Meditec, Albertslund, Denmark) was sprayed on the skin and the rat was wrapped in plastic drape (Vita wrap; Good Year, Svendborg, Denmark). Using an aseptic operative technique, a 20 mm ventral midline incision was made in the skin of the abdomen and the stomach was mobilized. A polyethylene catheter (i.d. 0·78 mm, o.d. 1·22 mm) with a 1 mm collar at 10 mm from the distal part of the catheter was introduced into the forestomach through a puncture and sutured (4-0 silk suture) in the serosa and musculature around the catheter collar. Using a 100 mm cannula, the catheter was guided through the left side of the abdominal muscle layer approximately 20 mm from the incision, under the skin to the back and out between the scapulas. The midline incision was closed in layers using silk suture (4-0); the procedure took about 30 min. The procedure is a modification of the technique described by Tsukamoto *et al.* (1984).

A rat 'jacket' (Alice King Chatham; Medical Arts, Hawthorne, CA, USA) was fitted to take the strain from the spring coil and swivel. The rat was then housed in a plexiglas metabolism cage and the catheter flushed with 1 ml water once a day. A 6 d period was allowed for recovery after the operation and acclimatization to the metabolism cage in a thermostatically-controlled room with a 12 h artificial light–dark cycle. In this period the rats had free access to water and standard rat pellets (Altromin Werke, Lage, Germany). The rats lost an average of 15 g BW during the first three postoperative days and then started to gain weight. On day 7 the experiments were performed as described below.

The rats were killed by exsanguination. Inspection of the abdominal cicatrice and catheter placement was performed to ensure the absence of infection.

Experimental protocol

Expt 1. On day 7 the rat pellets were removed and the rats were either starved for 4 d or given a 19 h/d continuous infusion of a liquid diet (17.00 to 12.00 hours) for the next 4 d. Urine was collected daily on days 8–11 into plastic vessels containing 5 ml 2 M-HCl and stored at −20° until analysis. ^{15}N-glycine infusion, as described below, was started on day 10 and urine was collected for measurement of isotope enrichment on day 11.

The diets were isoenergetic with the same carbohydrate (CHO):fat ratio, and were given as 1 MJ/kg BW per d with 9·1 g protein/kg BW per d when containing protein (Table 1). To ensure that observed differences were not due to different series of rats, only two or three out of the six rats in each series were given the same diet. The protein-free diet was made

Table 1. *Expt 1. Composition (% energy) of the diets*

Diet*	Percentage of energy as			
	Protein	CHO	Fat	MCT:LCT
Protein-free	0	65	35	0:100
Intact casein†	15	55	30	34:66
Hydrolysed soya-bean protein	15	55	30	50:50
Hydrolysed casein‡	12	53	35	14:86

CHO, carbohydrate; MCT, medium-chain triacylglycerols; LCT, long-chain triacylglycerols.
* The energy content of all diets was 4·20 kJ/ml.
† Casein plus lactalbumin.
‡ Casein plus whey protein.

of maltodextrin (Malto-Energi; Jensen Clinical Nutrition Service, Dianalund, Denmark), long-chain triacylglycerol (LCT) oil (Lipofundin, 100 mg/ml, B. Braun Melsungen AG, Melsungen, Germany) and sterile water, but for technical reasons, without vitamins and minerals. The hydrolysed soya-bean protein diet was made from a commercially available formula diet (Top Up; Ferrosan Ltd., Copenhagen, Denmark) without modifications other than dilution with sterile water. This hydrolysate is produced by a mixture of bacterial proteases (MD Foods; Viby, J., Denmark). The intact casein diet was made from an intact casein formula diet (Salvimulsin MCT; Ercopharm Ltd, Vedbiek, Denmark) diluted with sterile water and supplemented with maltodextrin and LCT oil in order to obtain the same ratios of protein, fat and carbohydrate. The hydrolysed casein diet was made from a trypsin-digested casein-and-whey formula (Reabilan; Roussel Nutrition, Copenhagen, Denmark) diluted with sterile water. The protein, fat, CHO and amino acid compositions are shown in Tables 1 and 2. The tables reproduce the manufacturers' information and the modifications mentioned above. The protein content was measured by us.

Expt 2. From day 7 two rats were given hydrolysed soya-bean protein and three were given intact casein (same diets as in Expt 1). The rise in atom percent excess (APE) was followed for 24 h and therefore the feeding period was extended from 19 to 24 h/d, but with the same amount of protein and energy/d. Infusion of ^{15}N-glycine was started on day 10 as described below except for omission of the priming dose.

Expt 3. This experiment was performed because the diets in the first and second experiments had differences other than the protein sources. The hydrolysed soya-bean protein diet was the same as above. The hydrolysed casein diet was prepared by Ferrosan Ltd. from casein (without whey) which was hydrolysed by almost the same procedure that was used to hydrolyse the soya-bean protein (MD Foods). By molecular weight (Da) the distribution of peptides in the soya-bean hydrolysate was as follows: > 5000, 2%; 5000–3000, 1%, 3000–1000, 28%; 1000–500, 35%; 500–300, 18%; < 300, 16%. In the casein hydrolysate the distribution of peptides was: > 5000, 4%; 5000–3000, 11%; 3000–1000, 41%; 1000–500, 27%; 500–300, 10%; < 300, 8%. The protein-free diet was supplemented with L-methionine and L-tryptophan in the same amounts as the hydrolysed soya-bean protein and hydrolysed casein, and the diets contained the same CHO:fat ratio plus vitamins and minerals so that they differed only in the protein source.

After the pellets were removed on day 7, 4 d were allowed for assimilation to the new diet and 5 days were subsequently allowed for a more valid N balance, including faecal N, before the isotope infusion. The amount of faeces had been neglected in Expt 1, since faecal output was zero or near zero during the first 4 d with the liquid diets.

Table 2. *Amino acid composition of the diets (mg/g crude protein)*

	Intact casein	Trypsin-hydrolysed casein	Bacterial protease-hydrolysed	
			Casein	Soya-bean protein
Lys	77	85	67	68
Thr	49	44	35	39
His	26	25	28	24
Trp	15	18	16	11
Phe+Tyr	92	95	115	78
Met+Cys	32	39	29	34
Leu	111	101	107	67
Ile	60	51	41	41
Val	69	59	69	44
Ala	29	36	30	39
Glu	196	159	203	204
Asp	65	84	59	125
Pro	87	84	91	64
Arg	34	33	39	82
Gly	28	18	18	39
Ser	38	47	43	45

A 24 h continuous infusion of the liquid diets was employed in this experiment, as in Expt 2. Faeces and urine were collected on days 12–16 and stored at −20°. In this Expt it was also attempted to measure protein synthesis in tissues directly by the intravenous ^{3}H phenylalanine flooding-dose technique, but due to lack of experience the infusion was successful only in a fraction of the rats, in particular in only two of the rats given hydrolysed soya-bean protein. This was evident from low radioactivity in plasma samples, low or unmeasurable plasma and tissue concentrations of phenylalanine and low values for radioactivity in tissue proteins. The results are not shown. Blood samples were taken for determination of hormones and plasma amino acid composition. Feeding was continued until anaesthesia was introduced (Thiomebumal i.p.; 10 mg/100 g BW), i.e. about 30 min before killing. The last portion of urine for determination of ^{15}N (see below) was taken before the anaesthesia.

Measurement of protein turnover

A primed continuous intragastric infusion of ^{15}N-glycine (98 %, Sigma) was initiated 19 h before the end of the experiment. A bolus of 5·94 mg ^{15}N/kg BW was given over 5 min followed by a continuous infusion of 4·75 mg ^{15}N/kg BW per 24 h, added to the liquid diet. The ratio used between the doses of bolus and continuous infusion was the same as that used in human experiments (Jeevanandam *et al.* 1985) since we did not know beforehand the rate constant in rats. The results of Expt 2 showed that this did not affect the observed different effects of the proteins. The amount of ^{15}N-glycine administered during the continuous infusion was equal to about 8 % of the amount of glycine given in the dietary proteins. Urine was collected hourly during the period of plateau enrichment (15–19 h) for analysis of ^{15}N in urinary urea and ammonia.

A preinfusion urine sample was used to determine the ^{15}N background. Based on the method described by Jensen (1991), urinary ammonia was isolated by adding 4 ml saturated K_2CO_3 to a 4 ml portion of urine in a closed bottle and absorbing the evolved

ammonia on a 1/4 25 mm GF/C glass filter (Whatman) containing 50 μl 0·5 M-H_2SO_4. A diffusion time of 48 h was used which gave a recovery of about 90% of the ammonia. Urine (2 ml) was treated with 1 ml resin (Amberlite IR-120 Plus; Sigma) to remove ammonia, and subsequently incubated in a closed bottle with urease for 1 h at 37° and pH 6·5 to convert urea to ammonia. Then 4 ml saturated K_2CO_3 was added and ammonia was trapped as described above. After the diffusion procedure, filters were dried under atmospheric air for 1 h before they were placed in tin capsules. The samples were analysed using an elemental analyser (Carlo Erba NA1500) coupled to an isotope-ratio mass spectrometer (Finnigan MAT Delta; Jensen, 1991). Flux was calculated from the APE at plateau ^{15}N enrichment by the method of Picou & Taylor-Roberts (1969) by using the geometric average of APE in ammonia and urea (Fern *et al.* 1985*a*). Protein synthesis and degradation were calculated according to the equation:

$$Q = I + D = E + S$$

in which Q is flux, I is intake, D is degradation, E is excretion as urinary N and S is synthesis. The rates are expressed per kg final BW.

The procedures were approved by the Ethical Committee for laboratory animals in Denmark.

Analytical

N contents in urine, faeces and diets were measured by a micro Kjeldahl technique (Keltec System; Tecator AB, Hoganaes, Sweden). Urine was measured once, faeces and diet in duplicate. Plasma insulin and glucagon were determined by commercially available kits from Novo Nordisk Ltd., Copenhagen, Denmark, with intra-assay variations of 5·0 and 5·3% respectively. Corticosterone was measured by the kit supplied by Farmos Diagnostica, Turku, Finland with an intra-assay variation of 2·2%. IGF-1 in serum was determined after acid–ethanol extraction as described by Bang *et al.* (1991). Measurements of IGF-1 standard solutions in serum were parallel to the standard curve. The intra-assay variation was 3·9%. Due to large inter-assay variations each hormone was measured in all samples on the same day. Plasma amino acid composition was measured by the Pico-Tag® method, Waters Chromatography Division, Milford, Mass., USA.

Statistics

The data were analysed by one-way ANOVA. When significant differences were found, Tukey's procedure was used to make comparisons within a group with $P < 0·05$ considered significant. The results were presented as means with standard errors of the difference.

RESULTS

Expt 1

The protein-free diet improved N balance (i.e. intake (I) – urinary excretion (E); N in faeces was not measured; Table 3) compared with starvation. This was associated with a lower rate of excretion, increased protein synthesis and decreased degradation. N balance was positive and not significantly different with the protein diets. The amount of protein administered was intended to be equal in all three groups but the N content of the hydrolysed casein diet, measured after the experiment, turned out to be lower than stated by the manufacturer. In the protein-fed groups urea and ammonia accounted for 91% and 3% of total N excretion respectively, without differences between the groups.

Intact and hydrolysed casein increased protein synthesis 2·5- and 2·0-fold respectively, compared with the protein-free diet, and intact casein increased protein degradation 1·4-

Table 3. *Expt 1. Whole-body protein turnover in rats that were starved or given diets containing no protein, intact casein, hydrolysed casein or hydrolysed soya-bean protein**

(Mean values for four or five rats)

Diet	g protein/kg BW per d					
	I–E	Q_{geo}	I	D	E	S
Starved (*n* 5)	−6·35[a]	8·79[a]	0·00	8·79[bd]	6·35[bc]	2·44[a]
Protein-free (*n* 5)	−1·59[b]	6·51[b]	0·00	6·51[a]	1·59[a]	4·92[c]
Intact casein (*n* 4)	2·81[c]	18·32[c]	8·95[a]	9·37[bcd]	6·14[bc]	12·18[b]
Hydrolysed soya-bean protein (*n* 4)	1·99[c]	12·78[d]	9·07[a]	3·71[e]	7·08[b]	5·70[c]
Hydrolysed casein (*n* 4)	2·06[c]	15·78[e]	8·03[b]	7·75[d]	5·97[c]	9·81[d]
SED, n_x 4 & n_y 4	0·34	0·74	0·14	0·65	0·35	0·63
SED, n_x 4 & n_y 5	0·32	0·71	0·14	0·62	0·34	0·60
SED, n_x 5 & n_y 5	0·30	0·66	0·13	0·58	0·32	0·57

BW, body weight; I, protein intake; E, urinary nitrogen excretion × 6·25; Q_{geo}, flux; D, protein degradation; S, protein synthesis; SED, standard error of the difference between means.

[a,b,c,d,e] Mean values within each column with unlike superscripts were significantly different ($P < 0·05$).

* For details of diets and procedures, see Tables 1 and 2, and pp. 70–73.

fold. The difference between intact and hydrolysed casein was probably due to the lower protein administration in the latter group. Protein synthesis was not increased significantly by hydrolysed soya-bean protein but degradation was decreased by 43 %, compared with the protein-free diet.

When compared with hydrolysed soya-bean protein, intact casein increased protein synthesis and degradation 2·1- and 2·5-fold respectively. With hydrolysed casein the protein synthesis and degradation were 1·7- and 2·1-fold higher compared with hydrolysed soya-bean protein.

Table 4 shows flux calculated from APE of ammonia (Q_a) or from APE of urea (Q_u). Compared with starvation the protein-free diet decreased Q_a but not Q_u. Intact and hydrolysed casein increased both Q_a and Q_u more than did hydrolysed soya-bean protein.

Expt 2

Fig. 1 shows that the APE of both ammonia and urea were higher after about 4 h of isotope infusion with hydrolysed soya-bean protein compared with casein. With both proteins the APE in ammonia was higher than in urea. With casein the quasi-plateau enrichments were reached in about 5 and 8 h for ammonia and urea respectively, whereas the plateau enrichment was reached in about 11 and 18 h for ammonia and urea with hydrolysed soya-bean protein.

Expt 3

The protein diets gave almost the same positive N balance as in Expt 1, although in this experiment it was determined during the last 5 d of the 9 d experiment (Table 5; I–E in the table does not include faeces). Faecal N excretion was variable but similar in the three groups (equivalent to 0·40–0·49 g protein/kg BW per d). Before the operation the rats given the protein-free diet weighed 247 (SE 4) g. After 7 d with pellets *ad lib.* and 5 d with the protein-free diet, i.e. 4 d before termination of the experiment, the weight was 226 (SE 5) g and on the day of killing the weight was 218 (SE 6) g. The rats given the protein diets weighed 248 (SE 2) g before the operation, 4 d before termination they weighed 239 (SE 3) g

Table 4. *Protein synthesis and degradation calculated from atom percent excess (APE) in urinary ammonia and urea, in rats that were starved or given diets containing no protein, intact casein, hydrolysed casein or hydrolysed soya-bean protein**

(Mean values for four or five rats)

Diet	g protein/kg BW per d	
	Q_a	Q_u
Starved (n 5)	7·98[ad]	9·68[a]
Protein free (n 5)	4·83[b]	8·79[a]
Intact casein (n 4)	15·09[ce]	22·30[b]
Hydrolysed soya-bean protein (n 4)	9·98[de]	16·45[e]
Hydrolysed casein (n 4)	12·72[e]	19·65[d]
SED, n_x 4 & n_y 4	0·95	0·74
SED, n_x 4 & n_y 5	0·90	0·70
SED, n_x 5 & n_y 5	0·85	0·66

BW, body weight; Q_a, protein flux calculated from APE in ammonia; Q_u, protein flux calculated from APE in urea; SED, standard error of the difference between means.

[a,b,c,d,e] Mean values within each column with unlike superscript letters were significantly different ($P < 0·05$).

* For details of diets and procedures, see Tables 1 and 2, and pp. 70–73.

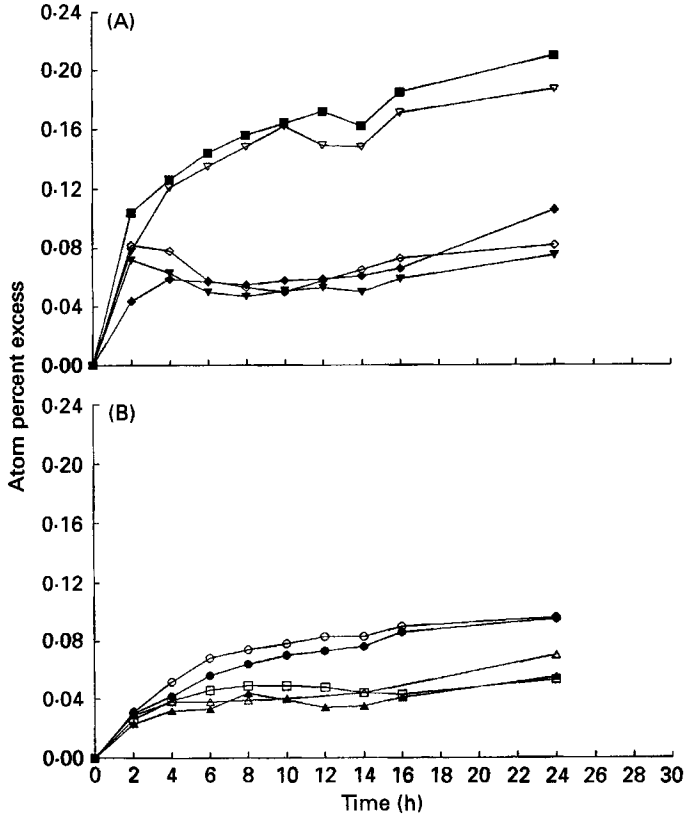

Fig. 1. ^{15}N enrichment of urinary ammonia (A) and urea (B) during continuous infusion of ^{15}N-glycine in two rats given hydrolysed soya-bean protein (○, ●, ■, ▽) and in three rats given intact casein. (△, ▲, ▼, □, ◇, ◆).

Table 5. *Expt 3. Whole-body protein turnover and plasma concentrations of hormones in rats given diets containing no protein, hydrolysed casein or hydrolysed soya-bean protein**

(Mean values for four to six rats)

Diet	g protein/kg BW per d						IGF-1 (μg/l)	Insulin (mU/l)	Glucagon (ng/l)	Cortico-sterone (μg/l)
	I–E	Q_{geo}	I	D	E	S				
Protein free (n 5)†	$-1{\cdot}88^{a}$	$8{\cdot}61^{a}$	0·00	$8{\cdot}61^{ab}$	$1{\cdot}88^{a}$	$6{\cdot}74^{ac}$	182^{a}	16	165	421
Hydrolysed casein (n 6)	$1{\cdot}81^{bc}$	$20{\cdot}49^{b}$	9·05	$11{\cdot}44^{a}$	$7{\cdot}24^{b}$	$13{\cdot}25^{b}$	433^{b}	22	216	452
Hydrolysed soya-bean protein (n 5)	$2{\cdot}38^{c}$	$15{\cdot}10^{c}$	9·10	$6{\cdot}00^{b}$	$6{\cdot}72^{b}$	$8{\cdot}39^{c}$	394^{b}	34	225	350
SED, n_x 4 & n_y 5							50	8	25	84
SED, n_x 4 & n_y 6							48	8	24	80
SED, n_x 5 & n_y 5	0·57	1·70	0·17	1·64	0·59	1·74				
SED, n_x 5 & n_y 6	0·54	1·63	0·16	1·57	0·57	1·67	45	7	23	75

BW, body weight; I, protein intake; E, nitrogen excretion × 6·25; Q_{geo}, flux; D, protein degradation; S, protein synthesis; IGF-1, insulin-like growth factor 1; SED, standard error of the difference between means.

[a,b,c] Mean values within each column with unlike superscript letters were significantly different ($P < 0{\cdot}05$).

* For details of diets and procedures, see Tables 1 and 2, and pp. 70–73.

† n 4 for hormones.

Table 6. *Expt 3. Plasma amino acid concentrations (μmol/l) during feeding, in rats given diets containing no protein, hydrolysed casein or hydrolysed soya-bean protein**

(Mean values for four to six rats)

Amino acid	Protein-free (n4)	Hydrolysed casein (n6)	Hydrolysed soya-bean protein (n5)	SED n_x 4 & n_y 6	SED n_x 4 & n_y 5	SED n_x 5 & n_y 6
Lys	567	668	632	66	69	62
Thr	65[a]	397[b]	354[b]	57	59	53
His	57	46	48	6	6	5
Trp	66	81	69	10	10	9
Met	35[a]	43	54[b]	7	7	6
Leu	80[a]	124[b]	104	11	12	11
Ile	37[a]	71[b]	95[b]	10	10	9
Val	90[a]	205[b]	174[b]	19	20	18
Ala	565[a]	379[b]	459	59	61	55
Glu	156[a]	81[b]	75[b]	21	22	20
Gln	659[a]	461[b]	439[b]	48	50	45
Asp	14	7	9	3	3	3
Asn	48	50	71	9	9	8
Pro	147[a]	245[b]	206	30	31	28
Arg	117[b]	120[b]	173[a]	15	16	14
Orn	46	31[a]	57[a]	8	9	8
Gly	380[a]	129[b]	202[b]	36	38	34
Ser	392[a]	151[b]	243[c]	31	33	29
Total	3521	3290	3465	265	275	249

SED, standard error of the difference between means.

[a,b,c] Mean values within each row with unlike superscript letters were significantly different ($P < 0.05$).

* For details of diets and procedures, see Tables 1 and 2, and pp. 70–73.

and on the day of killing they weighed 247 (SE 4) g. There was no difference in growth rate between the groups fed on the different proteins.

Hydrolysed casein increased synthesis 2·0-fold compared with the protein-free diet while degradation was not increased significantly. With hydrolysed soya-bean protein compared with the protein-free diet the effect on Q_{geo} was similar to that in Expt 1 but the 30% decrease in degradation did not reach statistical significance. As in Expt 1, hydrolysed soya-bean protein did not increase synthesis significantly. Hydrolysed casein compared with hydrolysed soya-bean protein gave a 1·6-fold higher rate of synthesis and a 1·9-fold higher rate of degradation.

Both proteins significantly increased the plasma concentration of IGF-1 while the changes in insulin, glucagon and corticosterone were insignificant.

Table 6 shows the changes in individual and total amino acids. Values for phenylalanine and tyrosine are not shown since they resulted from the (partially unsuccessful) ^{3}H-phenylalanine injection (see p. 72). The total concentration of other amino acids did not differ between the groups. Compared with the protein-free diet, hydrolysed casein significantly increased the concentrations of threonine, leucine, valine, isoleucine and proline, and decreased the concentrations of glutamate, glutamine, alanine, glycine and serine. The changes with hydrolysed soya-bean protein compared with the protein-free diet were similar except that arginine increased, methionine was significantly increased and the changes in alanine, proline and leucine were insignificant. With hydrolysed soya-bean protein compared with casein the mean concentration of all plasma amino acids, except

isoleucine and serine, changed in the direction that could be expected from the difference in amino acid composition of the proteins, but the difference was significant only for arginine, ornithine and serine.

DISCUSSION

The decrease in urinary N excretion caused by the protein-free diet compared with starvation (Table 3) was probably due to reduced gluconeogenesis from amino acids originating from endogenous proteins. This was associated with an increased reutilization of endogenous amino acids for protein synthesis (S/Q increased from 28% to 76%) and a decreased protein degradation as calculated from Q_{geo}. The latter effect, however, was largely due to a decrease in Q_a while Q_u was only marginally decreased (Table 4). In contrast to urea, only a limited number of amino acids contribute quantitatively to the formation of urinary ammonia. Glutamine has been estimated to contribute about 60% in the acidotic dog (Pitts, 1974) and in healthy human subjects (Jungas *et al.* 1992). The results suggest that energy supply did not decrease total amino acid flux (Q_u), i.e. protein degradation, but increased reutilization of liberated amino acids for protein synthesis at the expense of N excretion and formation of glutamine, decreasing Q_a.

The protein diets resulted in positive N balance. When faecal N is subtracted from the mean N balance (I–E) in Expt 3 (Table 5) the retention is about 1·7 g protein/kg BW per d. This would equal an expected weight gain of about 7–9 g/kg BW per d which compares well with the observed weight gain of about 8 g/kg BW per d. The aim of N balance was not achieved, possibly because the rats after the operation had only fully regained their weight loss towards the end of the experiment.

The difference in protein turnover between the protein-free and the starved state illustrates that it is not a trivial problem to select the control group when studying protein metabolism. The effect of casein was to increase synthesis but not degradation when compared with the starved state, while it increased both synthesis and degradation when compared with the protein-free state.

Our results suggest that the positive N balance achieved with intact or hydrolysed casein or hydrolysed soya-bean protein was obtained in two different ways: casein increased protein synthesis (and degradation) whereas soya-bean protein decreased protein degradation and did not significantly increase synthesis. The fact that the soya-bean protein was hydrolysed was not responsible for the difference since the effect of two different hydrolysed preparations of casein gave nearly the same effect as did intact casein.

The protein diets gave different rates of flux, depending on whether ammonia or urea was used for the calculation (Table 4), but the two proteins affected flux in the same direction whether expressed in terms of ammonia or urea.

According to the two-compartment model discussed by Fern *et al.* (1985*a*) the geometric fluxes presented for the protein diets in Table 3 are probably not more than about 10% different from the true values of N flux, since the $Q_a:Q_u$ ratio with the three protein diets varied from 0·61–0·68. The similarity of these ratios also indicates that the different effects of the proteins are not due to selective effects on flux of amino acids equilibrating with urinary ammonia compared with amino acids equilibrating with urea.

The ^{15}N-glycine method is an indirect method for measuring protein synthesis and degradation and, under basal feeding conditions, the ^{15}N-glycine method gives almost the same results as obtained with uniformly ^{15}N-labelled egg, yeast or wheat protein or with ^{14}C-labelled leucine (Golden & Jackson 1981; Fern *et al.* 1985*b*). However, with a change in conditions the assumptions of the method need to be reconsidered. According to the analysis of the method (Golden & Jackson, 1981) the higher APE with soya-bean protein could, rather than being due to effects on protein synthesis and degradation, be due to: (1)

the increased proportion of glycine and serine in hydrolysed soya-bean protein, (2) a possible increase in rates of glycine and serine *de novo* synthesis, or (3) to different degrees of exchange of ^{15}N from glycine to other amino acids. These changes could by themselves increase the APE of the end products and cause a decrease in calculated flux. We have evaluated these possibilities by adopting the principles of calculation described by Golden & Jackson (1981). In Expt 1 the measured APE of urea was 0·114 (SE 0·004) with intact casein while it was 0·167 (SE 0·006) with hydrolysed soya-bean protein. At rates for synthesis and degradation obtained with intact casein the 27% higher content of glycine and serine in soya-bean protein would itself increase the APE to 0·126. This means that $((0{\cdot}126-0{\cdot}114) \times 100/0{\cdot}167-0{\cdot}114) = 22\%$ of the difference between the two proteins can be explained by the difference in content of glycine and serine. An additional nearly 6-fold increase in *de novo* synthesis would be required to increase the APE to the measured value. Arnstein & Neuberger (1953) reported that the rate of *de novo* synthesis of glycine and serine in rats was independent of the content of these amino acids in the diet. In humans, *de novo* synthesis of glycine was unaffected by changing the amount of mixed amino acids in the diet but omission of non-essential amino acids reduced *de novo* synthesis by about 30% (Yu *et al.* 1985). Glucose infusion did not change *de novo* glycine synthesis in humans (Robert *et al.* 1982). From available evidence it seems that *de novo* synthesis of glycine and/or serine is rather constant during various experimental conditions and it is not obvious that soya-bean protein with a higher content of glycine + serine should increase *de novo* synthesis of these amino acids to the extent required to explain the difference in APE.

If adding to the effect of the increased dietary content of glycine and serine a change in the distribution of the dose of label from 50% in glycine and serine (which are assumed to be equally labelled) and 50% in all other amino acids (Golden & Jackson, 1981; Matthews *et al.* 1981) to an equal distribution in all amino acids, or to a distribution in glycine and serine only (Jackson & Golden 1980), calculated values of APE of 0·115 and 0·137 respectively are obtained. The pattern of distribution may change with the amount of ^{15}N-glycine administered and the infusion time (Jackson & Golden, 1980; Matthews *et al.* 1981) but the pattern does not change substantially under a variety of metabolic conditions such as feeding, fasting, refeeding, hypophysectomy, or treatment with growth hormone or corticotropin (Gaebler *et al.* 1959; Vitti & Gaebler, 1963; Jackson & Golden, 1980). It is not likely that the different proteins could produce highly different patterns of distribution, and even in the extreme case a change in distribution cannot fully explain the effect of soya-bean protein.

Despite these calculations it cannot be entirely ruled out that the indirect nature of the ^{15}N-glycine method and the disparate amino acid composition of the diets used led to artefacts that are more severe than we have been able to calculate. This could be elucidated by direct measurement of protein synthesis in individual tissues, as was attempted unsuccessfully with the flooding-dose technique in the last experiment.

Expt 2 (Fig. 1) showed that the difference between the proteins also existed without the priming bolus. The differences in time to reach the plateau enrichments agree with the differences in flux calculated from plateau enrichments (Table 4).

In Expt 3 the diets were identical except for the protein sources, and casein was hydrolysed almost by the same procedure that was used for hydrolysis of soya-bean protein. In this experiment the effects of the two proteins were approximately the same as in the previous experiments. The concentration of IGF-1 increased equally with the two proteins, as expected during protein feeding (Clemmons & Underwood, 1991). Also insulin, glucagon and corticosterone were similar with both proteins and the different effects on whole-body protein turnover cannot be explained by these hormones.

With hydrolysed casein there were increases in the plasma concentrations of all essential

amino acids (significant for branched-chain amino acids (BCAA) and threonine), compared with the protein-free diet. Garlick & Grant (1988) found that BCAA alone stimulated muscle protein synthesis *in vivo* in fasted rats to the same extent as did a complete mixture of amino acids, when infused together with glucose (which increased plasma insulin to the levels found in the present study). The stimulated protein synthesis with hydrolysed casein could be due to the content of BCAA and the fed-state concentrations of IGF-1 and insulin.

With hydrolysed soya-bean protein the conditions for stimulation of protein synthesis were apparently similar. Still, essential amino acids could be limiting for protein synthesis with hydrolysed soya-bean protein, due to the decreased degradation observed. With hydrolysed casein (Expt 3) the total availability of essential amino acids from the diet was 4·68 g/kg BW per d. By assuming 48 % essential amino acids in tissue proteins (value from food tables) an additional $(0{\cdot}48 \times 11{\cdot}44) = 5{\cdot}49$ g/kg BW per d would be available from degradation of endogenous sources. The protein synthesis observed with casein would require $(0{\cdot}48 \times 13{\cdot}25) = 6{\cdot}36$ g/kg BW per d as essential amino acids. Available essential amino acids were then used with an efficiency of $(6{\cdot}36 \times 100/4{\cdot}68 + 5{\cdot}49) = 63\,\%$. The same rate of synthesis would require an efficiency of $(6{\cdot}36 \times 100)/(3{\cdot}73 + 0{\cdot}48 \times 6{\cdot}00) = 96\,\%$ with the rate of degradation observed with hydrolysed soya-bean protein. Instead, an efficiency of 61 % was observed which is similar to the efficiency with casein. It may be that the primary effect of hydrolysed soya-bean protein was to inhibit protein degradation which led secondarily to decreased synthesis.

With casein the slight increase in protein degradation could be due to the decrease in the plasma concentration of most non-essential amino acids (significant for glutamate, glutamine, alanine, glycine and serine compared with the protein-free diet). Hydrolysed soya-bean protein had higher contents of aspartate, arginine, serine and glycine which were reflected in higher plasma concentrations of arginine, ornithine and serine (and insignificant increases for asparagine and glycine). It may be that the higher content of one or several of these amino acids could be responsible for the decreased degradation with soya-bean protein. Leucine (or α-ketoisocaproic acid) is probably the main regulatory amino acid for protein degradation in liver and muscle (Fulks *et al.* 1975; Mitch & Clark, 1984; Miotto *et al.* 1992) but, at least in liver, the non-essential amino acids glutamine, alanine and glycine further decrease protein degradation when leucine is present (Mortimore *et al.* 1989; Miotto *et al.* 1992).

This study demonstrates that two different dietary proteins induce different kinetics of ^{15}N-labelled glycine. This is associated with changes in the plasma amino acid composition that reflect the difference in the amino acid composition of the dietary proteins. The isotope results are not easily explained by differences other than the suggested changes in protein synthesis and degradation. However, the results should be verified by direct measurement of protein synthesis in individual tissues and an explanation for the observation should be verified experimentally.

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