Effects of long-term protein excess or deficiency on whole-body protein turnover in sheep nourished by intragastric infusion of nutrients

BY S. M. LIU, G. E. LOBLEY, N. A. MACLEOD, D. J. KYLE, X. B. CHEN AND E. R. ØRSKOV

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB

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The effect of long-term dietary protein excess and deficit on whole-body protein-N turnover (WBPNT) was examined in lambs nourished by intragastric infusions of nutrients. Ten sheep were given 500 mg N/kg metabolic weight ($W^{0.75}$) per d from casein for 2 weeks and then either 50 (L), 500 (M) or 1500 (H) mg N/kg$W^{0.75}$ per d for 6 weeks. Volatile fatty acids were infused at 500 kJ/kg$W^{0.75}$ per d. Daily WBPNT was measured by continuous intravenous infusion of [1-^{13}C]leucine 3 d before, and on days 2, 21 and 42 after the alteration in protein intake. Whole-body protein-N synthesis (WBPNS) was calculated as the difference between WBPNT and the protein-N losses as urinary NH_3 and urea. Whole-body protein-N degradation (WBPND) was then estimated from WBPNS minus protein gain determined from N balance. Fractional rates of WBPNS and WBPND were calculated against fleece-free body N content. WBPNS rates at the L, M and H intakes were respectively 35·1, 41·5 amd 63·7 g/d ($P < 0·001$) on average over the 6 weeks and WBPND rates were 39·5, 41·1 and 56·8 g/d ($P < 0·001$). The fractional rates of WBPNS were 5·01, 6·37 and 7·73% per d ($P < 0·001$) while those of WBPND were 5·64, 6·29 and 6·81% per d ($P < 0·005$) respectively. On days 2, 21 and 42, WBPNS rates at intake H were 54·0, 61·8 and 75·4 g/d ($P = 0·03$) respectively, and WBPND rates were 43·2, 56·4 and 70·9 g/d ($P = 0·03$); at intake L the amounts were 38·2, 34·2 and 32·8 g/d for WBPNS ($P = 0·003$) and for WBPND were 43·4, 38·0 and 36·9 g/d ($P = 0·016$) respectively. There were no significant ($P > 0·05$) differences in fractional rates of WBPNS and WBPND with time at either the L or H intake. We concluded that absolute protein turnover was affected both by dietary protein intake and by body condition while the fractional rate of turnover was predominantly influenced by intake.

Whole-body protein turnover: Intragastric nutrient infusion: Sheep

Whole-body protein turnover (WBPT) includes dynamic flows of both endogenous and exogenous substrates and exceeds considerably the net N balance (NB) or protein gain. Both WBPT and NB vary with dietary intake at any given metabolic and physiological state (Waterlow *et al.* 1978; Garlick, 1980; Riis, 1983; Lobley, 1993). Whole-body protein synthesis (WBPS) and degradation (WBPD), the constituent processes of WBPT, can vary in similar or opposite directions but usually a high protein intake results in elevated WBPT accompanied by an improved growth rate. Conversely, a low WBPT usually stems from a low intake, with fasting as an extreme. Identifying the influences of both intake and the metabolic state on protein metabolism in ruminants is, however, complicated since experiments involving adaptation to protein over- or under-supply in ruminants have to overcome three concerns. The first, which is exclusive to normally fed ruminants, involves the difficulty in quantifying the actual protein supply because rumen fermentation alters both the quantity and quality of absorbed dietary amino acids. This is overcome by the technique of intragastric infusion which allows control of both energy and amino acid

supply and at a constant rate, aiding the establishment of a metabolic steady state preferred for protein kinetic studies. The second involves resolution of acute from chronic effects. This is best achieved by within-animal comparisons and use of the non-destructive irreversible loss rate procedure (Reeds & Lobley, 1980), allowing measurements at different time intervals. The third problem relates to changes in body condition, consequent on the differences in nutrient supply, which possibly have an effect on protein metabolism. Such changes may be assessed, at least for protein mass, by the use of continuous NB and extrapolation data from terminal slaughter.

These approaches were combined in the present study with sheep to examine the effect of both protein intake and body condition on protein metabolism. The energy supply from volatile fatty acids (VFA) was maintained constant while protein infusion was varied to either 3, 1 or 0·1 times N equilibrium over a 42 d period and changes in NB, WBPS and WBPD were monitored at various intervals.

MATERIALS AND METHODS

Animals

Ten Suffolk × Greyface castrated lambs (initial live weight 36·9 (SD 7·9) kg; this includes one replacement animal of 22 kg) of 7 months of age were surgically prepared with a permanent rumen cannula and an abomasal catheter as described previously (Ørskov *et al.* 1979).

Treatments

The lambs were housed in metabolism cages. They were nourished completely by intragastric nutrient infusions as described by Ørskov *et al.* (1979) and MacLeod *et al.* (1982). The mixture of VFA contained acetic acid, propionic acid and butyric acid in the molar proportions of 0·65:0·25:0·10, and was infused initially at approximately 100 kJ/kg metabolic weight ($W^{0·75}$) per d and increased to 500 kJ/kg$W^{0·75}$ per d over a period of 12 d. This infusion was then maintained at a constant rate throughout the experiment. The casein infusion started at a rate of 250 mg N/kg$W^{0·75}$ per d and was increased to 500 mg N/kg$W^{0·75}$ per d over a period of 6 d. These levels of casein and VFA were maintained for 2 weeks (maintenance period). The lambs were then divided into three groups and infused with casein-N at 1500 (H, *n* 3), 500 (M, *n* 3) and 50 (L, *n* 4; one lamb died at day 35) mg/kg$W^{0·75}$ per d respectively for a treatment period of 6 weeks. The amounts of casein infused were calculated according to the initial live body weight and maintained at a constant rate over the period although the live weight did change with time.

Procedures for infusion of [1-^{13}C]leucine

Each sheep was fitted with two polyvinyl catheters in the external jugular veins and maintained as described previously (Lobley *et al.* 1992). Each lamb was infused with labelled leucine on four occasions: 3 d before the alteration in protein intake (in the maintenance period) and on days 2, 21 and 42 after alteration of protein intakes. Infusates were prepared with 0·3 g [1-^{13}C]leucine (99 atoms %; Tracer Technologies Inc., Somerville, MA, USA) dissolved in 200 g sterile saline (9 g NaCl/l) and infused via the jugular catheter for 8 h (20 g/h; 250 μmol leucine/h).

During each period of leucine infusion a total of six blood samples (each of 5 ml) was withdrawn into EDTA: one was taken pre-infusion to establish plasma-free leucine natural abundance while the remainder were taken at hourly intervals over the final 4 h infusion. Plasma was prepared immediately by centrifugation at 3000 *g* for 15 min at 4° and then stored at −20°.

Daily NB measurements were performed throughout (Ørskov & MacLeod, 1982). Urine samples were stored at −20° for analysis of total N, urea and NH_3.

Chemical analysis

[1-^{13}C]4-methyl-2-oxopentanoate (MOP) and [1-^{13}C]leucine fractions from 0·7 ml plasma were prepared and analysed for enrichment as described by Calder & Smith (1988), using a gas chromatography mass spectrometer system (VG TRIO-1; VG MASSLAB Ltd, Manchester, Ches.).

Urine N was measured by a Kjeldahl procedure (Davidson *et al.* 1970) while urea and NH_3 were measured by modified automated procedures from Marsh *et al.* (1965) and Fawcett & Scott (1960) respectively.

Total body N content was determined at the end of the experiment when the animals were shorn and killed, and the digesta were removed. The fleece-free empty body was minced, weighed, sampled and then freeze-dried. The N concentrations of the samples were determined by the Kjeldahl procedure. The fleece was dried at 100° and the N content calculated by multiplying the dry weight by 0·134 (Agricultural Research Council, 1984), Since the sheep was sheared at the beginning of the experiment, the fleece-N content was considered as the N retained in wool growth over the experimental period. Daily NB (N intake minus faeces-N and urine-N) was then adjusted by subtraction of the calculated daily average N retention in the fleece. The fleece-free body-N (FFBN) content throughout the experiment was then calculated from extrapolation of the terminal N content by subtracting the cumulative adjusted NB.

Calculations

In this experiment the irreversible loss rate (ILR) of free leucine was calculated based on plasma MOP enrichments as representative of the tissue precursor pool (Matthews *et al.* 1982). The ILR was calculated as follows:

$$\text{ILR (mmol/h)} = \left(\frac{\text{[1-}^{13}\text{C]leucine enrichment in infusate}}{\text{[1-}^{13}\text{C]MOP enrichment in plasma}} - 1\right) \times \text{[1-}^{13}\text{C]leucine infusion rate (mmol/h).}$$

The ILR was then converted to the N flux of the whole-body free amino acids (N ILR, g/d) assuming body protein contains 16% N and 6·8% leucine (Reeds & Lobley, 1980). The calculation for whole-body protein-N synthesis (WBPNS, g/d) was based on a simplified model (Waterlow *et al.* 1978) as follows:

$$\text{N ILR} = \text{WBPNS} + \text{O} = \text{I} + \text{WBPND},$$

where O is amino acid-N oxidation and I is dietary amino acid-N intake (g/d). In the present study O was assumed as the sum of the daily urinary excretion (g/d) of urea-N and NH_3-N (Reeds *et al.* 1980). Whole-body protein-N degradation (WBPND, g/d) was calculated as the difference between WBPNS and the daily adjusted NB. The fractional rates of N ILR, WBPNS and WBPND were calculated against the corresponding FFBN content.

In such procedures direct measures are not always possible and the current study involved determination of leucine plasma ILR, NB, urinary urea-N and NH_3-N, and terminal body-N content. These data were then used to derive N ILR, WBPNS, WBPND and FFBN. Such procedures are widely adopted but care must be exercised that compound errors do not arise.

Statistical analysis

To reduce differences between the animals they were all initially maintained at 500 mg N/kg$W^{0.75}$ per d for 2 weeks. A treatment (protein intake) effect was calculated as the difference between the average over days 2, 21 and 42 and the value at the maintenance period. The treatment effect of each animal was then used in one-way analysis of variance with intake as factor to derive the difference between the three N intakes. On the other hand, the effect of the body condition was derived by a comparison of the differences between days 2, 21 and 42 at the same intake. Since the comparisons were made within animals, the original values were used directly. Two-way analysis of variance was used with time as factor and animal as a blocking factor. One lamb on the low N intake died at day 35 so that the results on day 42 were not obtained. The missing values were estimated by Genstat 5 and were included in the means but excluded in variance analyses. All analyses were performed using Genstat 5 (1988).

RESULTS

At the end of the experiment, live weights of the sheep averaged 44·3, 33·3 and 37·7 kg for the H, M and L intakes respectively. The changes over the 6 weeks were +7·3, +1·3 and −2·5 kg respectively.

N ILR, protein synthesis and degradation

ILR of N, urinary urea-N and NH_3-N, WBPNS, WBPND and adjusted NB are shown in Table 1. During the maintenance period all these variables were similar for all animals.

Responses to alteration in N intake were rapid and persistent. Average N ILR values were 78·0, 46·0 and 37·8 g/d for H, M and L intakes respectively. The urinary urea-N plus NH_3-N excretions were 14·2, 4·5 and 2·7 g/d. Calculated WBPNS rates were 63·7, 41·5 and 35·1 g/d and WBPND rates were 56·8, 41·1 and 39·5 g/d, while daily NB values were 6·9, 0·5 and −4·4 g/d respectively. The average changes after alteration in N intake are listed in Table 3. The differences were all significant ($P < 0.001$) between intakes.

N ILR, WBPNS and WBPND at either the high or the low intake were significantly different between days 2, 21 and 42, indicating the persistent effect of long-term protein excess or deficit on protein metabolism.

At the higher intake when compared with the maintenance period the daily N ILR increased by 51%, WBPNS by 36% and WBPND by 20%. The converse occurred for the low-protein animals: N ILR decreased by 32%, WBPNS by 30% and WBPND by 21%.

Fractional rates of N ILR, WBPNS and WBPND

Total FFBN content, fractional rates of N ILR, WBPNS and WBPND are shown in Table 2 and the average differences in values between the treatment and maintenance periods are listed in Table 3. The fractional rates of N ILR at the H, M and L N intakes averaged 9·42, 7·04 and 5·39% per d respectively over the 6 weeks. The fractional synthesis rates (WBPNS) were 7·73, 6·37 and 5·01% per d, and the fractional degradation rates (WBPND) were 6·81, 6·29 and 5·64% per d respectively. Dietary N intake significantly influenced the fractional rates of N ILR ($P < 0.001$) and WBPNS ($P < 0.005$) but not of WBPND ($P > 0.1$). On average, raising N intake from the initial 500 mg/kg$W^{0.75}$ per d to treatment H induced increases in the fractional rate of N ILR of 27%, WBPNS of 14% and WBPND of 0·2%, while a decrease of N intake from the initial 500 mg/kg$W^{0.75}$ per d to treatment L resulted in reductions of 22, 21 and 11% respectively. Thus, change in WBPND between the L and H N intakes was 11%, approximately one third of the alteration in WBPNS.

Table 1. *Whole-body amino acid-N irreversible loss rate (N ILR), protein-N synthesis (WBPNS) and degradation (WBPND), and nitrogen balance (NB) in sheep infused with different amounts of casein and with 500 kJ/kg metabolic weight ($W^{0.75}$) per d of volatile fatty acids for 6 weeks**

	N intake (mg/kg$W^{0.75}$ per d)		
	1500	500	50
n...	3	3	4§
N ILR (g/d)			
Maintenance†	52·1	46·2	55·8
Day 2	64·7	46·7	41·9
21	78·8	45·3	36·6
42	90·4	46·0	34·6
SED (for time)	4·8	2·3	2·5
P value‡	0·006	NS	0·001
Urinary urea-N plus NH_3-N (g/d)			
Maintenance	4·7	4·5	5·2
Day 2	10·7	5·5	3·7
21	17·0	3·8	2·4
42	14·9	4·0	1·8
SED (for time)	1·6	0·7	0·4
P value‡	0·008	NS	0·001
WBPNS (g/d)			
Maintenance	47·4	41·7	50·6
Day 2	54·0	41·2	38·2
21	61·8	41·5	34·2
42	75·4	41·9	32·8
SED (for time)	4·9	2·1	2·6
P value‡	0·03	NS	0·003
N balance (g/d)			
Maintenance	−0·3	0·3	−0·1
Day 2	10·8	−0·3	−5·2
21	5·4	0·9	−3·8
42	4·5	0·8	−4·1
SED (for time)	0·6	0·3	0·2
P value‡	0·001	NS	0·001
WBPND (g/d)			
Maintenance	47·7	41·4	50·7
Day 2	43·2	41·6	43·4
21	56·4	40·6	38·0
42	70·9	41·1	36·9
SED (for time)	5·0	2·2	2·7
P value‡	0·03	NS	0·016

* For details of procedures, see pp. 830–832.

† The maintenance period (500 mg N/kg$W^{0.75}$ per d for all animals). None of the variables was significantly different.

‡ The effect of duration of time at the same N intake. The residual degrees of freedom were 6, 6 and 7 on the high, medium and low N intakes respectively.

§ See statistical analysis, p. 832.

Table 2. *Fleece-free body-N (FFBN) content, fractional rates of amino acid-N irreversible loss rate (N ILR), protein-N synthesis (WBPNS) and degradation (WBPND) in sheep infused with different amounts of casein and with 500 kJ/kg metabolic weight ($W^{0.75}$) per d of volatile fatty acids for 6 weeks**

	N intake (mg/kg$W^{0.75}$ per d)		
	1500	500	50
FFBN content (g)†			
Maintenance	705	641	802
Day 2	716	642	796
21	845	653	709
42	931	670	639
SED (for time)	12	3	4
P value‡	0·001	NS	0·001
N ILR (% per d)			
Maintenance	7·44	7·22	7·02
Day 2	9·19	7·27	5·34
21	9·36	6·98	5·32
42	9·72	6·87	5·50
SED (for time)	0·72	0·33	0·35
P value‡	NS	NS	NS
WBPNS (% per d)			
Maintenance	6·77	6·51	6·37
Day 2	7·66	6·43	4·86
21	7·39	6·40	4·99
42	8·13	6·28	5·19
SED (for time)	0·70	0·23	0·34
P value‡	NS	NS	NS
WBPND (% per d)			
Maintenance	6·82	6·46	6·39
Day 2	6·08	6·47	5·55
21	6·73	6·26	5·57
42	7·63	6·15	5·81
SED (for time)	0·70	0·28	0·36
P value‡	NS	NS	NS

* For details of procedures, see pp. 830–832.

† FFBN content on day 42 was measured and the remainder were extrapolated from the 42 d content and cumulative N balance.

‡ The effect of duration of time at the same N intake. The residual degrees of freedom were 6, 6 and 7 on the high, medium and low N intakes respectively.

After alteration to the H or L intake there were no significant differences ($P > 0·05$) in any of the fractional rates between days 2, 21 and 42.

Contributions to N ILR and protein synthesis

The ILR of leucine consists of the amino acids ingested from the diet (exogenous) and those degraded from body protein (endogenous); the latter can be estimated from the difference between flux and intake (infused casein). During the maintenance period the exogenous N supply was 7·0 g/d while the corresponding N ILR was 51·8 g/d. The endogenous contribution was thus 44·8 g/d or 86% N ILR. On day 2 after alteration to the H intake the exogenous supply was 22·1 g/d while the endogenous was 42·6 g/d or 66% N ILR. At the L intake the values were 0·8 g/d (exogenous) and 41·2 g/d (endogenous, 98% N ILR) respectively. The quantities of the endogenous contributions were not significantly different

Table 3. *Treatment (N intake) effects calculated as the average over days 2, 21 and 42 minus the value at the maintenance period**

	N intake (mg/kgW$^{0.75}$ per d)				
	1500	500	50	SED	*P* value†
N ILR (g/d)	25·9	−0·2	−18·0	3·3	< 0·001
Urinary urea-N plus NH_3-N (g/d)	9·5	−0·1	−2·6	1·3	< 0·001
WBPNS (g/d)	16·3	−0·1	−15·5	3·9	< 0·001
N balance (g/d)	7·2	0·2	−4·3	0·8	< 0·001
WBPND (g/d)	9·1	−0·3	−11·2	3·8	< 0·005
FFBN content (g)	125·8	14·2	−86·8	10·9	< 0·001
N ILR (% per d)	2·0	−0·2	−1·6	0·5	< 0·001
WBPNS (% per d)	1·0	−0·1	−1·4	0·5	< 0·005
WBPND (% per d)	−0·01	−0·2	−0·8	0·5	> 0·1

N ILR, whole-body amino acid-N irreversible loss rate; WBPNS, whole-body protein-N synthesis; WBPND, whole-body protein-N degradation; FFBN, fleece-free body-N, W$^{0.75}$, metabolic weight.

* For details of experimental protocol, see pp. 830–832.

† The residual degree of freedom was 7.

from that at maintenance, although N ILR changed in proportion to the intakes. From days 2 to 42, however, either a progressive increase ($P = 0·006$) or a decrease ($P < 0·001$) in N ILR dependent on N intake was observed (Table 1) and, because the exogenous intake was constant, time-related changes in the endogenous contribution must therefore have occurred.

During the maintenance period the proportion of N ILR from synthesis was 0·91 (SE 0·01) but after the alteration in N intake, the contributions were 0·82, 0·89 and 0·93 at the H, M and L N intakes respectively. Differences between the treatments were significant ($P < 0·001$) but independent of time.

N retention and protein synthesis

The average daily NB and corresponding protein-N synthesis values were significantly and positively correlated (r 0·725, n 38, $P < 0·001$). The slope was 0·313 (SE 0·05, $P < 0·001$) g N retained/g protein-N synthesized.

DISCUSSION

Effect of protein intakes

The effects of dietary protein intakes on whole-body protein metabolism of the sheep in the present experiment agree with other published reports. The average changes from the N intake of 500 mg/kgW$^{0.75}$ per d in the maintenance period to 1500 mg/kgW$^{0.75}$ per d were +36% in protein synthesis and +20% in degradation. Changes in synthesis and degradation between the maintenance intake and the low N intake of 50 mg/kgW$^{0.75}$ per d were −30 and −21% respectively. The effect of N intake on protein synthesis was thus greater than the effect on degradation. This was also observed in growing young sheep fed on 0·6, 1·2 and 1·8 × energy maintenance (M) diets where protein synthesis and degradation increased by 25 and 18% between 1·2 × M and 1·8 × M, but decreased by 25 and 16% respectively from 1·2 × M to 0·6 × M (Harris *et al.* 1992). In steers reduced from maintenance to fasting, protein synthesis decreased by 18% and protein degradation by 2%; while between maintenance and 1·6 × M protein synthesis and degradation increased

by 26 and 20% respectively (Lobley *et al.* 1987). Thus, overall response of degradation to a change in dietary protein intake is smaller than that of synthesis.

Acute responses to dietary protein changes

Marked acute responses in protein synthesis and degradation were observed on day 2 after alteration in intakes although they were not statistically different from the maintenance because of large individual variation, indicative of a rapid adjustment in metabolism to exogenous protein intake. The reaction to excess protein involved a stimulation of synthesis and inhibition of degradation (Table 1). A similar suppression has been observed in rat tissues. For example, inhibition of liver protein degradation measured by the decay of $NaH^{14}CO_3$ was found during the first few days of regeneration in partly hepatectomized rats (Swick & Ie, 1974; Scornik & Bothol, 1976), and immediately after feeding (Garlick, 1980). Rapid regrowth of muscle protein was accompanied by a substantial suppression of protein breakdown (Young *et al.* 1971). In contrast, when the sheep were switched from maintenance to low protein status there were immediate declines in total protein flux and in both synthesis and degradation, and because the fractional rates established by day 2 were similar to those on days 21 and 42 the adjustment was thus both rapid and substantial. This was also reflected in urinary total-N excretion which fell by 20% on the first day of intake reduction but then declined more slowly and by a similar absolute percentage over the following 41 d.

The differences in fractional synthesis rates between the excess and deficit protein intakes may represent similar changes in the turnover of all body tissues; alternatively, some organs may be more responsive than others. The latter option is more probable because for sheep the peripheral tissues, skin and skeletal muscle, exhibit marked changes in fractional synthesis rate in response to overall changes in dietary status (Oddy *et al.* 1987; Lobley *et al.* 1992; see Lobley, 1993) whereas liver and the gastrointestinal tract are affected only slightly (Lobley *et al.* 1994).

Effects of long-term protein excess and deficiency

Time-related increases and decreases in daily absolute protein turnover in the lambs were observed for the high and the low N intakes. Since the N intakes were constant these changes could only arise from altered endogenous contributions, i.e. body protein degradation. However, neither WBPNS (% per d) nor WBPND (% per d), changed significantly with time, indicating that the rate of protein metabolism per unit of protein mass, once established within the first 2 d of resetting the intakes, was not altered. Therefore, the time-related changes in turnover, and in turn synthesis and degradation, must have occurred predominantly from changes in body-protein mass since the synthesis:turnover ratio was also time-independent. In fact, the body-protein mass was enlarged by 34% or diminished by 21% over the 6 weeks as the consequence of constant excess or deficit in protein intake. A close relationship between the increased body protein content and the leucine flux was observed in growing lambs on a normal diet with or without casein infusion (Davis *et al.* 1981). Body condition also affected protein turnover in children, before and after recovery from malnutrition states. During malnutrition a lower rate of protein turnover (g/kg live weight per d) was observed compared with that after the recovery even at comparable intakes (Waterlow *et al.* 1977).

Partition of precursors between synthesis and oxidation

In the simplified model described in the calculation, amino acid turnover is partitioned between protein synthesis and oxidation, and this partition adjusts to different nutritional conditions. In the present experiment the changes in N ILR, WBPNS and urea-N plus

NH_3-N were −32, −30 and −48% when N intake was altered from 500 mg/kg$W^{0.75}$ per d to 50 mg/kg$W^{0.75}$ per d; the corresponding values when N intake was raised to 1500 mg/kg$W^{0.75}$ per d were +51, +36 and +201% respectively. Greater changes in oxidation compared with synthesis, particularly on a high N intake, were therefore observed. This is also reported in other studies. For example, the increments in leucine oxidation and protein synthesis in sheep offered either 0·6× or 1·8× maintenance energy diets were 190 and 71% respectively (Harris *et al.* 1992). In heifers fed at either 1·2× or 2·4× maintenance energy, leucine oxidation and protein synthesis increased by 88 and 58% respectively (Hammond *et al.* 1987). Differential sensitivities are also shown in response to altered protein level; for example, in growing pigs fed on diets containing 150 and 290 g crude protein/kg, leucine oxidation and protein synthesis changed by +111 and +11% respectively (Fuller *et al.* 1987), while in obese human subjects maintained on a protein-free diet for 3 weeks leucine oxidation and protein synthesis decreased by 83 and 39% respectively (Garlick *et al.* 1980). Regulation of amino acid oxidation provides a major mechanism by which animals adapt to different dietary nutrient intakes, i.e. output adjusts to input (Waterlow *et al.* 1978).

It is not well understood how the partition of amino acids between synthesis and oxidation is controlled by the body. Protein synthesis is universal for all tissues but the major catabolic site for most amino acids is the liver, with the notable exception of the branched-chain amino acids (see Harris & Lobley, 1991). Oxidation is probably associated with substrate concentration (Waterlow *et al.* 1978; Lindsay, 1980; Motil *et al.* 1981; Nissen & Ostaszewski, 1985; Hammond *et al.* 1987) and enzymes regulating the catabolism of amino acids have Michaelis constants (K_m) in the millimolar range while for enzymes initiating protein synthesis (e.g. t-RNA synthetases) the values are in the micromolar range. Thus at low concentration a greater proportion of amino acids will react with the synthetases. As the synthetic pathway of protein becomes saturated and amino acid concentrations increase, the catabolic pathway will assume greater importance (see Lindsay, 1980). Since the K_m of many of the amino acid transaminases (the usual first step in amino acid catabolism) is greater than the amino acid concentrations *in vivo*, these enzymes are sensitive to increased amino acid concentrations and hence oxidation is stimulated (see Waterlow *et al.* 1978). With a low protein intake or amino acid input the plasma (and tissue) concentrations of amino acids often remain low, and so does the oxidation of labelled amino acids (Motil *et al.* 1981; Fuller *et al.* 1987). With intakes in excess of the maintenance requirement the concentrations increase sharply and the increased enzyme activity results in an increased rate of oxidation.

CONCLUSION

Overall, the results of the experiment suggested that daily protein turnover, synthesis and degradation during long-term protein excess or deficiency were affected by both dietary protein intake and body protein condition, i.e. body protein mass. At constant dietary intakes the rate of protein turnover per unit of body-protein mass was rapidly established, with the values mainly determined by dietary intake.

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