Urea synthesis and leucine turnover in growing pigs: changes during 2 d following the addition of carbohydrate or fat to the diet

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1. Studies have been made of the time-sequence of protein metabolic and hormonal changes following an abrupt increase in carbohydrate or fat intake. [^{3}H]leucine and [^{14}C]urea were infused for 72 h, via the aorta, into fourteen female pigs (30–38 kg body-weight). At 24 h after the start of the infusion their feed was either changed to one of two isonitrogenous diets containing additional starch (group BS, five animals) or fat (group BF, five animals), or remained unaltered (group BB, four animals). The distribution space of urea was measured by the dilution of a single dose of [^{14}C]urea given both 48 h before and 48 h after the change in diet. The changes in the concentration and specific radioactivity of blood leucine were used to infer changes in protein turnover and those of plasma urea to measure total amino acid catabolism. The concentrations of blood glucose and plasma insulin and cortisol were also measured at approximately two-hourly intervals for the 48 h period following the change in diet.

2. Within 4 h of either change in diet blood leucine concentration was lowered and the leucine specific radioactivity was raised above that in group BB, but after 24 h both the concentration and specific radioactivity of leucine returned to values similar to those in group BB. Eventually the blood leucine specific radioactivity was slightly but not significantly reduced below that of group BB.

3. The addition of starch to the diet significantly reduced the synthesis and concentration of urea within 4 h but, although the addition of fat to the diet eventually lowered the urea concentration and synthesis, both changes were delayed for 18–24 h.

4. In group BS plasma glucose and insulin rose after the addition of starch, but after 24–36 h both returned to values that were the same as those in the animals that received the same diet throughout (group BB). The addition of fat to the diet altered neither blood glucose nor plasma insulin concentrations. The addition of either carbohydrate or fat to the diet eventually reduced plasma cortisol concentrations, but the change did not occur until 24 h after the change in diet.

5. The results suggest that alterations in non-protein energy supply exert their immediate effect on the degradation of whole-body protein. They do not exclude the possibility that these early changes may reflect opposing changes at different sites. The results also suggest that the rate of urea synthesis may be controlled by the balance between the concentrations of insulin and cortisol, but that under the conditions of these experiments there was little relation between these hormones and the turnover of body protein as measured by the turnover of blood leucine.

The addition of non-protein energy substrates to the diets of young animals increases the rate of nitrogen retention (Munro, 1964; Fuller & Crofts, 1977) and additions of carbohydrate or fat appear to be equally effective in this respect (Forbes *et al.* 1939; Nakano *et al.* 1973; Reeds *et al.* 1981). Previous measurements of the changes in protein metabolism suggested that the increased rate of protein deposition under these circumstances was associated with increased protein synthesis, decreased protein degradation and reduced amino acid catabolism (Reeds *et al.* 1981). These studies did not shed any light on the mechanisms whereby these effects were achieved and they did not establish whether one of these processes was primarily responsive to dietary energy supply (the others changing perhaps as part of a secondary adaptation) or whether there were simultaneous changes in all processes.

In an attempt to examine these possibilities the time-course of the protein metabolic changes has been followed by measuring the alterations in urea synthesis and leucine turnover, using the former to measure amino acid catabolism and the latter to measure body protein turnover. In addition, the changes in plasma insulin and cortisol

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concentrations that occur when the diet is abruptly supplemented with either carbohydrate or fat were measured.

MATERIALS AND METHODS

Animals and diets. The experiments were carried out with fourteen female pigs, the progeny of a Large White boar and Large White x Landrace sows. Two diets of high energy:protein value were prepared by adding either 264 g starch (diet S, five pigs; group BS) or 143 g lard (diet F, five pigs; group BF) to 1 kg of a basal cereal–fish meal diet (diet B, four pigs; group BB) described in detail in Reeds *et al.* (1980). The diets were offered once hourly from an automatic feeder at daily rates of 90 (basal; group BB), 124 (group BS) and 113 (group BF) g/kg metabolic body-weight (kg body-weight$^{0.75}$). The mean body-weights and the intakes of apparently digestible N and energy in each study are given in Table 1 (p. 305). Although the supplemented diets were intended to be isoenergetic, some small loss of energy on pelleting of diet S occurred.

Experimental design. When the animals weighed about 20 kg two PVC catheters (NT2; Portex Ltd, Hyde, Kent) were inserted into the aorta via the superficial saphenous arteries (Fuller *et al.* 1977). After the catheterization the animals were offered the basal diet *ad lib.* until they achieved a body-weight of 28 kg. At this time they were transferred to metabolism crates and offered the same diet, once hourly, at 90 g/kg body-weight$^{0.75}$. When they weighed approximately 32 kg the amount of diet B was adjusted to give 1·1 MJ/kg body-weight$^{0.75}$ at the weight that it was estimated that they would reach 4 d later (i.e. at the mid-point of the study). A bladder catheter was inserted and complete collections of urine and faeces were begun. After 2 d, in order to measure the urea distribution space, the animals were injected via the distal aortal catheter with approximately 10 μCi [^{14}C]urea (Amersham International plc, Amersham, Bucks; CFA 41; 5 μCi/ml in aqueous sodium chloride (9 g/l)). Blood samples were taken from the proximal aortal catheter at 1, 2, 5, 10, 30, 60, 120, 180, 240 and 300 min after the injection. At 10.00 hours on the next day a constant infusion of [^{14}C]urea (approximately 2 μCi/h) and L-[4,5-^{3}H]leucine (Amersham International plc; TRK 170; 10–15 μCi/h) each in NaCl (9 g/l; 0·37 ml/h) was begun and maintained for the next 68 h.

After 5–6 h of infusion (15·00–16·00 hours, day 1) two blood samples (5 ml) were taken for the measurement of the specific radioactivity of blood leucine. These were used to enable comparisons to be made with earlier studies of leucine turnover in animals receiving the basal diet. After 22 h of infusion (08.00 hours on day 2) a further four blood samples (10 ml) were taken at 30-min intervals. After the last of these samples had been taken (10.00 hours, day 2) the animals were offered either diet S (group BS), diet F (group BF) or continued to receive the same diet (group BB).

Blood samples (10 ml) were then taken every 2 h for the next 8 h, at 22.00 hours on day 2, at two-hourly intervals between 08.00 and 22.00 hours on day 3 and between 08.00 and 10.00 hours on day 4. The infusion was then terminated, blood samples were taken at hourly intervals and after 3 h, a second injection of [^{14}C]urea (approximately 10 μCi) was administered and blood samples were taken, as before, for a second determination of the urea distribution space.

Sample preparation and analysis. Each 10 ml blood sample was mixed immediately with 200 I.U. heparin and 2000 I.U. of the proteolytic inhibitor Trasylol (Sigma Chemical Co., Poole, Dorset). Of this mixture 5 ml were added immediately to an equal volume of 1 M-perchloric acid (PCA) containing 2·5 μmol L-norleucine, mixed and placed in ice-water. After 15 min this sample was centrifuged at 2000 ***g*** for 15 min and the supernatant fraction stored at −20° until analysed. A 0·5 ml portion of the blood was diluted with an equal volume of water and stored at −20° until analysed for glucose content (Trinder, 1969). The remainder was centrifuged at 3000 ***g*** for 15 min, 1 ml of the plasma was mixed with 10 ml

NE 260 scintillant (A & J Beveridge, Edinburgh) and the remainder was stored in 1 ml portions at −20° until analysed for urea (March *et al.* 1965), insulin (Bassett & Thorburn, 1971) and cortisol (Amerlex Kit; Amersham International plc).

The concentration and specific radioactivity of free leucine in blood was measured as described previously (Reeds *et al.* 1980). The specific radioactivity (SR) of plasma urea was taken as

$$\text{SR} = \frac{^{14}\text{C (disintegrations/min per ml plasma)}}{\text{urea (mg/ml plasma)}}, \quad (1)$$

Preliminary measurements had shown that urea was the only detectable source of ^{14}C.

The radioactivity in the samples was measured in a Packard 460CD scintillation spectrometer using the external-standard channels ratio method for quench correction. The average efficiencies of ^{3}H and ^{14}C counting were 28 and 65% respectively.

Calculations

Urea pool size and turnover. Initial analysis of the semi-logarithmic plot of urea specific radioactivity *v.* time showed that, as observed by Bruckental *et al.* (1980), extrapolation to zero time of the linear portion of the curve led to a clear overestimate of the urea distribution space. When calculated in this way the urea distribution space exceeded body-weight in a number of cases. The calculations were therefore based on the two-compartment model used by Bruckental *et al.* (1980) in their description of blood urea kinetics in cattle. The change in the urea specific radioactivity with time was fitted to a curve with the general equation:

$$\text{SR}_t = H_1 e^{-g_1 t} + H_2 e^{-g_2 t}, \quad (2)$$

where t is the time (h) after the injection of urea, H_1 and H_2 are the apparent pool sizes with rate constants (h) of turnover of g_1 and g_2 respectively. H_1 and H_2 were calculated as described by Shipley & Clark (1972). The sum $(H_1 + H_2)$ was taken as the urea distribution space. When the injection of [^{14}C]urea followed a constant infusion of [^{14}C]urea the rate of fall of [^{14}C]urea specific radioactivity during the 3 h between the end of the infusion and the injection of urea was measured. The measured specific radioactivity of [^{14}C]urea after the injection was adjusted by subtracting the contribution of residual [^{14}C]urea predicted from the rate of fall before the injection.

The urea entry rate under steady-state conditions (i.e. 08.00–10.00 hours days 2 and 4 in groups BS and BF) was taken as:

$$\text{urea entry rate} = \frac{\text{rate of [}^{14}\text{C]urea infusion}}{\text{urea specific radioactivity at plateau}}.$$

During the adaptive period, i.e. when the urea pool was not in a steady-state, the rate of entry was calculated as follows:

$$\text{average entry rate between } t_1 \text{ and } t_2 = \frac{r - \frac{1}{2}(P_1 + P_2)[(\text{SR}_1 - \text{SR}_2)/(t_1 - t_2)]}{\frac{1}{2}(\text{SR}_1 + \text{SR}_2)}, \quad (3)$$

where r is the rate of infusion, P_1 and P_2 are the calculated pool sizes at times t_1 and t_2 respectively and SR_1 and SR_2 are the respective specific radioactivities at t_1 and t_2 respectively. The rate of urea excretion in groups BS and BF was measured over the 48 h period before the change in diet and over the 2nd to 3rd day after the change in diet.

Leucine turnover. In the steady-state the flux of leucine was calculated as described previously (Reeds *et al.* 1980). Under non-steady-state conditions, eqn (3) could not be used to calculate the apparent rate of blood leucine turnover largely because valid estimates of the free leucine pool size could not be obtained. An additional complication arose because,

in the control studies, the label re-entry from the breakdown of body protein led to a significant rise with time in the specific radioactivity of blood leucine. Thus, after 72 h of infusion the specific radioactivity was about 50% higher than the value obtained after 6 h (see also Schwenk *et al.* 1985). Therefore in groups BS and BF any changes in leucine specific radioactivity were superimposed on a continually rising 'baseline'.

An attempt was made as follows to predict the time-related change in leucine specific radioactivity that would have occurred in groups BS and BF had no dietary change been imposed on the animals. At each time-point after 08.00 hours on day 2 (i.e. after 22 h of infusion) the predicted leucine specific radioactivity (SR_t*) was calculated as:

$$SR_t^* = (1-e^{-k_f t}) + (1-e^{-k_p t}) + (0{\cdot}85\, I_t.K_p^*). \quad (4)$$

In this equation k_f (the rate constant of turnover of the free leucine pool) was taken as 50/d (Reeds *et al.* 1980) and k_p (the rate-constant of body protein turnover) was calculated as

$$\frac{\text{amino-N flux} - \text{urea synthetic rate}}{\text{calculated body protein mass}}.$$

The baseline NH_2-N flux was calculated (Reeds *et al.* 1980) from the specific radioactivity of blood leucine after 5–6 h of leucine infusion. Body protein mass was calculated from the relation between body water (calculated from the urea distribution space) and body protein (log protein $= -0{\cdot}785 + 1{\cdot}156$ (log water); Kotarbińska, 1969). Calculated values of k_p ranged, in the basal state, from 0·07 to 0·11 d.

I_t was the dose of labelled leucine infused between the start of the infusion and time t and K_p* was the calculated proportion of body protein degraded over each interval of iteration (0·1 h). The value 0·85, used to scale the term for recycled label ($I_t.K_p$*), was the proportion of the dose of leucine incorporated into body protein in the basal state (i.e. 1 − proportion of dose catabolized). When compared with the leucine specific-radioactivity in group BB, values calculated with eqn (4) predicted over 90% of the variations with time.

$$\text{Predicted SR} = (\text{observed SR} \times 0{\cdot}88) + 0{\cdot}22;\ (r^2\, 0{\cdot}91;\ \text{df}\, 46).$$

Statistics

The significance of differences between means was estimated by two-tailed Student's t test, using paired values for changes with time within a dietary group and using group means and standard deviations for comparisons between groups in the steady-state. In Table 4 (p. 307) the statistical significance of the change in leucine specific radioactivity was calculated from the difference between the observed specific radioactivity and that predicted by eqn (4).

RESULTS

The body-weights of the animals and their daily intakes of metabolizable energy and apparently digestible N are shown in Table 1. Between the first and second measurements of the urea distribution space the daily weight gains of the animals were 560, 675 and 700 g/d in groups BB, BF and BS respectively. The addition of either carbohydrate or fat to the diet lowered the excretion and total synthesis of urea (Table 2) and while these changes were accompanied by a reduction both in the plasma urea concentration and in the body pool of urea they were not associated with a change in the apparent distribution space of urea.

In group BS the rate of urea synthesis was reduced significantly by at least 4 h after receiving additional carbohydrate (Table 3) and this new, lower rate was maintained thereafter (Tables 2 and 3). In group BF the eventual fall in urea synthesis (Table 2) did

Table 1. *Body-weights (W; kg), the intakes of apparently digestible nitrogen (ADN; g N/kg $W^{0.75}$) and apparently digestible energy (DE; MJ/kg $W^{0.75}$) of fourteen pigs at the start and end of a 4 d period during which they received a basal diet throughout (group BB), a basal diet for 2 d, and a high-carbohydrate diet for 2 d (diet S; group BS) or a basal diet for 2 d and a high-fat diet for 2 d (diet F; group BF)*†

(Mean values together with a common estimate of variance, pooled standard deviation (PSD))

Group	Diet‡	*n*	W (kg)§	ADN (g N/kg $W^{0.75}$)	DE (MJ/kg $W^{0.75}$)
BB	Basal	4	33·2	2·13	1·11
	Basal		35·2		
BS	Basal	5	34·0	2·07	1·10
	Diet S		36·8	2·18	1·42**
BF	Basal	5	34·4	2·07	1·10
	Diet F		37·1	2·07	1·59**
PSD			3·2	0·09	0·07

Mean value was significantly different from that for basal intake of DE: ** $P < 0.01$.

† For groups BS and BF the intakes of energy and N are for the separate 2 d periods and for the whole 4 d period for group BB.

‡ For details of diets, see p. 302 and Reeds *et al.* (1981).

§ The 'basal' value represents the value immediately before the first injection of [^{14}C]urea.

Table 2. *The daily rates of urea excretion and synthesis (g nitrogen/kg body-weight $(W)^{0.75}$), the body urea pool size (g urea/kg W) and urea distribution space (l/kg W) of fourteen female pigs receiving either the basal diet throughout a 4 d period (group BB) or the basal diet for 2 d followed by diets containing additional carbohydrate (diet S; group BS) or fat (diet F; group BF) for 2 d*‡

(Mean values together with a common estimate of variance, pooled standard deviation (PSD))

Group	Diet	*n*	Urea (gN/kg $W^{0.75}$) Excretion	Urea (gN/kg $W^{0.75}$) Synthesis	Pool size	Distribution space
BB	Basal	4	0·92	1·02	0·225	0·712
	Basal			0·96	0·213	0·696
BS	Basal	5	0·93	1·00	0·244	0·720
	Diet S		0·68*†	0·73*†	0·182*†	0·692
BF	Basal	5	0·93	1·03	0·247	0·703
	Diet F		0·60*†	0·63*†	0·150**†	0·688
PSD			0·08	0·05	0·015	0·013

Mean values were significantly different within a group (paired *t* test): * $P < 0.05$, ** $P < 0.01$.

Mean values were significantly different from group BB: † $P < 0.05$.

‡ For group BB the values for urea excretion are for the whole 4 d period and for urea synthesis the average for the 3 d period of [^{14}C]urea infusion. For groups BS and BF the initial rate of urea excretion is for the first 2 d and the final is that measured over the 4th and 5th days of receipt of the high-energy diets. In these groups urea synthesis is quoted for the periods 08.00–10.00 hours on days 2 and 4.

§ For details of diets, see p. 302 and Reeds *et al.* (1981).

Table 3. *Changes with time in the apparent rate of urea synthesis and the concentration of plasma urea over a 2 d period during which fourteen female pigs were offered the basal diet throughout (group BB) or were offered (at 0 h) diets containing additional carbohydrate (group BS) or fat (group BF)*†

(Values are expressed as a proportion of the average value between −2 and 0 h together with a common estimate of variance, pooled standard deviation (PSD) for four animals in group BB and five animals in groups BS and BF)

Group...	BB		BS		BF	
Time period (h)	Urea concentration	Urea synthesis	Urea concentration	Urea synthesis	Urea concentration	Urea synthesis
−2	1·02	0·98	0·99	1·02	1·00	0·99
−1	1·00	1·00	1·02	0·91	1·02	1·00
0	1·00	1·03	1·03	1·03	1·00	1·04
2	1·05	0·98	0·97	0·86	1·04	1·06
4	0·95	0·97	0·92	0·69*	0·98	1·08
6	1·02	0·97	0·90	0·88*	0·99	1·12*
8	1·02	0·99	0·86	0·64**	0·92	1·11*
10	1·04	0·94	0·85*	0·77**	0·99	1·09
22	0·98	0·98	0·81*	0·89*	0·84*	0·66**
24	0·98	0·98	0·87	0·73**	0·71**	0·73**
26	0·96	1·04	0·88	0·77*	0·68**	0·58**
28	0·99	0·99	0·80*	0·80*	0·66**	0·53***
30	1·06	0·97	0·82	0·64**	0·64**	0·67**
36	1·00	0·97	0·80*	0·70*	0·68**	0·67**
46	0·95	0·98	0·79*	0·68**	0·62***	0·66**
48	1·01	1·01	0·79*	0·73*	0·62**	0·61**
PSD	0·05	0·11	0·09	0·11	0·10	0·16

Mean values were significantly different (paired *t* test; df 3) from the mean value between −2 and 0 h: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† For details of diets, see p. 302 and Reeds *et al.* (1981).

not occur until at least 24 h after changing the diet (Table 3) and for the first 12 h was increased, significantly so at 6 and 8 h after adding fat to the feed.

In the control animals (group BB) the concentration of leucine was constant throughout the infusion period but when either carbohydrate or fat was added to the diet there was a reduction, commencing about 2 h after the change in the diet, in the concentration of blood leucine (Table 4). This reduced concentration was maintained for about 12 h in group BF and then rose to attain control values 26 h after the introduction of the fat-supplemented diet (diet F). By the end of the infusion the concentration of blood leucine was the same irrespective of the diet.

Whether directly compared with the values for group BB or with those calculated from eqn (4) the initial fall in leucine concentration for groups BS and BF was accompanied by an increase in the leucine specific radioactivity in the blood (Table 4). The subsequent rise in the concentration of leucine was associated with a fall in the leucine specific radioactivity and eventually the blood leucine specific radioactivity for groups BF and BS was slightly, but not significantly, lower than the value for group BB. During the initial phase of the adaptive period the product of leucine concentration and specific radioactivity (a measure of the rate of leucine removal from the blood) was unaltered, although during the later phase the value was lower, suggesting a more rapid rate of labelled leucine disposal.

Table 4. *Changes with time in the concentration (C) and specific radioactivity (SR) of blood leucine over a 2 d period during which fourteen female pigs received the same diet throughout (group BB; four animals) or were offered (at 0 h) diets containing additional carbohydrate (group BS; five animals) or fat (group BF; five animals)*§

(The values are expressed as a proportion of the values obtained between −2 and 0 h together with a common estimate of variance, pooled standard deviation (PSD) for four animals in group BB and for five animals in groups BS and BF)

Group...	BB		BS		BF	
Time period (h)	C	SR	C	SR	C	SR
−2	1·00	0·96	1·02	0·99	1·02	1·00
−1	1·00	1·00	1·02	0·99	0·98	0·97
0	0·99	1·01	0·98	1·04	1·02	1·01
2	1·01	1·04	0·90†	1·06	0·95	1·09
4	0·96	1·15	0·76‡	1·20	0·93	1·17
6	1·02	1·19	0·68‡	1·43*	0·80‡	1·33*
10	0·96	1·27	0·86†	1·56*	0·74‡	1·58*
22	0·97	1·37	0·92†	1·47	0·86†	1·30
24	1·01	1·33	0·93	1·52	0·90†	1·25
26	1·08	1·43	0·97	1·35	1·04	1·24
28	1·01	1·45	0·99	1·19	0·91	1·33
30	1·00	1·50	0·98	1·20*	0·99	1·37
36	0·91	1·48	1·10	1·28	1·06	1·36
46	1·00	1·52	0·99	1·34	0·97	1·42
48	1·04	1·54	0·97	1·44	1·01	1·46
PSD	0·02	0·13	0·03	0·12	0·02	0·14

Mean values were significantly different (df 3) from that predicted from eqn (4) (see p. 304): * $P < 0·05$.
Mean values were significantly different (df 7) from that for group BB at the same time-point: † $P < 0·05$, ‡ $P < 0·01$.
§ For details of diets, see p. 302 and Reeds *et al.* (1981).

Table 5. *Concentrations of blood glucose (mmol/l), leucine (mmol/l), plasma urea (mmol/l), insulin (mU/l) and cortisol (μg/l) in fourteen female pigs at the beginning and the end of a 4 d period in which they received a basal diet throughout (group BB) or the basal diet for 1 d and diets containing additional carbohydrate (diet S; group BS) or fat (diet F; group BF) for a further 3 d*

(Values are means with 1 SEM for four animals in group BB and for five animals in groups BS and BF and are based on the average concentrations in four samples taken between 08.00 and 10.00 hours on days 2 and 4)

			Glucose		Leucine		Urea		Insulin		Cortisol	
Group	Diet†	*n*	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
BB	Basal	4	6·38	0·17	0·271	0·008	5·27	0·56	24	2	26	2
	Basal		6·40	0·18	0·276	0·009	5·10	0·53	23	3	29	2
BS	Basal	5	6·23	0·06	0·298	0·009	5·72	0·20	24	2	30	3
	Diet S		6·29	0·08	0·280	0·011	4·55*	0·11*	24	1	15*	2
BF	Basal	5	6·26	0·10	0·270	0·005	5·89	0·39	26	2	26	2
	Diet F		6·56	0·24	0·271	0·008	3·71*	0·24*	23	3	13*	2

Mean values were significantly different within a group (paired *t* test; df 3): * $P < 0·001$.
† For details of diets, see p. 302 and Reeds *et al.* (1981).

Table 6. *Changes with time in the concentration of blood glucose, plasma insulin and cortisol and the ratio, insulin:cortisol (I:C) over the 2 d period during which the animals were offered (at 0 h) diets containing additional carbohydrate (group BS) or fat (group BF)*†

(Values are expressed as a proportion of the mean values between −2 and 0 h together with a common estimate of variance, pooled standard deviation (PSD) for five animals in each group)

Time period (h)	Group BS				Group BF			
	Glucose	Insulin	Cortisol	I:C	Glucose	Insulin	Cortisol	I:C
−2	1·01	0·98	0·94	1·00	1·02	0·95	1·02	0·98
−1	0·98	1·01	1·06	0·98	1·01	1·08	0·90	1·02
0	0·99	1·01	1·00	1·01	0·97	0·98	1·08	1·00
2	1·12*	1·27*	1·09	1·28*	1·02	0·95	0·98	1·03
4	1·11*	1·91**	0·99	2·00**	0·97	0·99	1·42*	0·64*
6	1·13*	1·41**	1·10	1·32*	1·00	1·08	1·26	0·91
8	1·09	1·25	1·06	1·21*	0·98	1·05	1·05	1·02
10	1·08	1·10	0·73*	1·31*	0·96	1·03	0·89	1·23
22	1·11	1·62**	0·80*	2·35**	1·02	1·04	0·95	1·02
24	1·14	1·55**	0·58**	2·60**	1·01	0·99	0·67**	1·48*
26	1·05	1·32*	0·57**	2·53**	1·02	0·89	0·72*	1·27*
28	1·05	1·19	0·60**	1·98**	1·03	1·08	0·72*	1·53**
30	1·05	0·98	0·51**	1·68**	1·09*	1·21	0·71*	1·60**
36	1·03	0·94	0·61**	1·54**	1·09*	0·99	0·66**	1·41*
46	1·02	0·98	0·62**	1·85**	1·07	1·01	0·41**	1·59*
48	1·02	1·02	0·42**	2·00**	1·04	0·85	0·64**	1·76**
PSD	0·04	0·08	0·08	0·12	0·03	0·13	0·09	0·11

Mean values were significantly different from the mean value between −2 and 0 h: * $P < 0·05$, ** $P < 0·01$.
† For details of diets, see p. 302 and Reeds *et al.* (1981).

For groups BB and BF there was no significant change in the concentration of either blood glucose or plasma insulin at any time (Tables 5 and 6). In group BS the ingestion of a high-carbohydrate diet (diet S) was associated with a transient rise in both blood glucose and plasma insulin concentrations but again both returned to their initial values by the end of the infusion (Tables 5 and 6). By the end of the infusion the concentration of plasma cortisol was significantly reduced in both the experimental groups but was unaltered in group BB (Table 5) so that by the end of the infusion period the ratio, insulin:cortisol was significantly increased in both experimental groups. Once again a diet-related difference in the time-course of the change in insulin:cortisol was evident, with the ratio rising early (as a result of the rise in plasma insulin) in group BS but only after 24 h in group BF. In the latter group the rise in insulin:cortisol resulted entirely from the reduced concentration of plasma cortisol.

DISCUSSION

Interpretation of isotopic values in the non-steady-state

While a temporary perturbation in an existing metabolic steady-state may be a necessary part of metabolic adaptation it complicates the calculation of 'instantaneous' rates of metabolite entry and exit to and from the blood. In their analysis of the treatment of experiments similar to these, Shipley & Clark (1972) concluded that in order to draw valid conclusions during the non-steady-state it is necessary for the time between samples to be short in relation to the initial rate-constant of turnover of the appropriate metabolites and for accurate estimates of the changes in the pool size of the metabolite to be obtained.

These criteria could be met with urea. In the present studies the half-life of the body urea

pool was about 5 h, while the half-life of equilibration was approximately 5–10 min. Samples separated by 1–2 h were sufficiently frequent to define the rate of change of urea specific radioactivity. The urea pool size also could be estimated by standard isotope-dilution techniques, although admittedly during the non-steady-state, the urea pool size had to be calculated from the product of the urea distribution space (1/kg body-weight), the plasma urea concentration (g/l) and the estimated body-weight (kg) at each time-point. We assumed that weight gain was linear between the beginning and the end of each infusion period and while this appeared to be valid in general it may have introduced some additional error.

Neither criterion could be met with leucine. First the half-life of the blood leucine pool is about 15 min, requiring too-frequent sampling, and second the amino acid does not equilibrate in body water in the same way as urea, so that isotope dilution is of dubious validity in the estimation of the body free leucine content. The interpretation of the information on free leucine labelling therefore has to remain qualitative and the possibility of artefacts arising from transient changes in the distribution of free leucine between the extracellular and intracellular phases should be borne in mind.

Changes in protein metabolism

In group BS the rapid and coordinated changes in leucine entry rate (suggestive of a rapid fall in body protein degradation) and urea synthesis (reflecting a reduction in amino acid catabolism) would have been sufficient to have maintained an elevated rate of N retention. Even so there was a transient reduction in leucine concentrations. In group BF although there was an initial reduction in amino acid entry there was no immediate reduction in the rate of amino acid catabolism. Although this combination would have sustained a transient rise in protein deposition this would have been at the expense of a large reduction in free amino acid concentrations. The fact that this did not occur might suggest that protein synthesis also fell. Indeed during the first 12 h after fat supplementation urea synthesis rose and presumably N retention temporarily was poorer than that during the preceding period. The basis of this observation (see also Munro, 1964) is unknown but might relate to the adaptations in energy metabolism that occur when dietary energy supply is increased (Reeds *et al.* 1981). In group BF an increased turbidity in the plasma was evident within 4 h of introducing the high-fat diet and presumably an increased rate of fat absorption had occurred by this time. In these animals the increased energy intake required both an increase in total energy expenditure and an alteration in intermediary metabolism to enable the additional fat to be metabolized efficiently. The animals in group BF had previously received a high-carbohydrate diet and it is possible that during the early period of adaptation the capacity for fatty acid metabolism in the liver was insufficient to dispose completely of any additional hepatic fat load and that hepatic acetyl-CoA levels may have risen. If so, one consequence might have been a transient rise in the concentration of acetyl-glutamate in the liver leading to a transient activation of carbamoyl phosphate synthase and hence of urea synthesis (Stewart & Walser, 1980; Walser & Stewart, 1981). Only when both energy expenditure and the capacity of the liver to metabolize fat rose was the anabolic stimulus of the additional energy intake fully realized and reflected in a reduction in the rate of urea synthesis.

Despite the differences in urea synthesis the changes in leucine concentration and labelling were essentially the same in both experimental groups. In the past the reduction in amino acid concentrations that follow carbohydrate ingestion (Lotspeich, 1947; Munro, 1964; Reeds, 1972) have been assumed to result from an increase in tissue protein synthesis. Recent work (Garlick *et al.* 1983) has not substantiated this view and the present results suggest that the early reduction in blood amino acid concentrations after the ingestion of

higher amounts of carbohydrate and fat was caused by a reduction in the rate of amino acid entry into the blood. Assuming that there had not been a temporary slowing of amino acid absorption from the gut, the findings suggest that the immediate whole-body protein metabolic response to both carbohydrate and fat was a reduction in body protein degradation and perhaps also in protein synthesis. The rise in body protein synthesis was a later event with both diets. It should be emphasized, however, that changes in splanchnic protein synthesis could, by being derived from leucine of a lower specific radioactivity than arterial leucine, have resulted in a rise in arterial specific radioactivity. Indeed it could be argued that changed splanchnic metabolism may well dominate the whole-body changes at early times after the change in diet.

Factors affecting protein metabolism

Under some circumstances glucose, free fatty acids and amino acids can influence tissue protein synthesis and degradation in vitro (Fulks *et al.* 1975; Nakano & Ashida, 1975; Buse & Reid, 1975; Morgan *et al.* 1980; Tischler *et al.* 1982; Mortimore & Poso, 1984). It has, however, proved difficult to reproduce these effects in vivo (McNurlan *et al.* 1982; Garlick *et al.* 1985). In the present work the transient rise in blood glucose in group BS was only 10% of the basal concentration and the concentration of leucine (an amino acid often invoked as an important influence on protein turnover) was initially reduced.

In the present work we concentrated our measurements on insulin and cortisol as there is much evidence to implicate both in the physiological control of protein synthesis and degradation, especially in skeletal muscle (Manchester, 1970; Waterlow *et al.* 1978; Garlick *et al.* 1983; Millward *et al.* 1983; Odedra *et al.* 1983).

Insulin levels were little altered from the basal (diet B) values once the adaptation to either of the high-energy diets had been completed but the hormonal environment had been altered towards more anabolic balance by a rise in insulin:cortisol resulting from a fall in plasma cortisol. The time at which the ratio rose was different between the diets and with both diets the rise in insulin:cortisol correlated in time with the fall in urea synthesis. These results suggest a causal relation between the rise in the insulin:cortisol ratio and the fall in the rate of urea synthesis, but the hormonal changes did not clearly correlate with the changes in leucine concentration and turnover. The factor that controls this aspect of the protein metabolic adaptation to increased dietary energy remains obscure.

The results of these experiments suggest that although the eventual changes in protein metabolism that underlie the 'protein-sparing' effects of dietary carbohydrate and fat are similar, the route by which they are achieved and the rate at which they occur are different. Both carbohydrate and fat appear to cause a rapid reduction in amino acid entry into the blood and we interpret this as suggesting that their initial effect in the whole body is on protein degradation. It must be recognized that changes in the distribution of free leucine may have influenced these results. Furthermore the measurements represent the resultant of changes in protein metabolism in different tissues and the apparently bi-phasic response may represent different time-courses of the changes in visceral and peripheral tissue protein turnover. To some extent the results demonstrate the limitation of 'simple' whole-body amino acid turnover measurements. A fuller understanding of the mechanism of the effect of dietary non-protein energy on N retention may require the application of techniques that allow the non-destructive measurement of protein synthesis and degradation in anatomically defined tissues (e.g. Oddy & Lindsay, 1986).

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