Effects of changes in the intakes of protein and non-protein energy on whole-body protein turnover in growing pigs

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1. The relationships between the intakes of protein and of non-protein energy (NPE), nitrogen retention and body protein synthesis have been studied in female pigs weighing 30 and 35 kg.

2. Four animals were assigned to three regimens and given a conventional (basal) diet supplemented with fat, carbohydrate or protein. After 1 week, measurements of N excretion in urine and faeces (7 d collection) and gaseous exchange (3–4 d) were made. At the end of the balance period a solution of [1-^{14}C]leucine was infused at a constant rate. Body protein synthesis was then calculated as the difference between the apparent irreversible loss of blood leucine and the loss of ^{14}C in expired air.

The animals were then offered the basal diet without supplement for 10 d and the measurements of N retention, energy retention and protein synthesis were repeated.

3. The intakes of metabolizable energy (ME; MJ/kg body-weight (W)$^{0.75}$ per d) were 1·75 for fat, 1·58 for carbohydrate, 1·25 for protein and 1·18 for the basal diet; corresponding intakes of apparently digestible N (ADN; g N/kgW$^{0.75}$ per d) were 2·30, 2·31, 4·35 and 2·17. Daily N retention, which during the period of basal feeding was 13·6 g was increased by between 3·4 and 7·2 g by the supplements. Daily fat deposition was also increased in the animals that received the diets supplemented with carbohydrate and fat.

4. The rate of leucine catabolism was significantly reduced in the animals receiving the diets that were supplemented with NPE and increased by the addition of protein to the diet.

5. When based on the specific radioactivity of blood leucine both the synthesis and breakdown of body protein (per unit metabolic body-weight) were increased by 30% in the animals receiving the high-protein diet but the increases in protein synthesis associated with the addition of carbohydrate (+14%) and fat (+12%) were much less marked. Consideration of these results together with previous observations (Reeds *et al.* 1980) suggested that body protein synthesis (g N/d) increased by 0·88 for each g increase in daily ADN and by 0·93 for each MJ increase in daily ME intake.

6. Comparison of the results obtained with the animals given high-carbohydrate diets and those given high-protein diets suggested an increase in heat production of 14 KJ/g of additional fat deposition. A similar comparison of animals receiving the high-protein and basal diets suggested a heat increment of 23·5 KJ/g additional protein deposition. The changes in heat production and protein synthesis in the animals given the protein supplement were compatible with a heat increment of 5·3 KJ/g additional protein synthesized. Because of the large proportion of heat production associated with the deposition of fat this could not be confirmed with either of the other supplements, but it is possible that the energy cost of protein accretion varies with the relative proportions of protein and NPE in the diet.

At a fixed intake of dietary protein the rate of nitrogen retention is dependant on the amount of non-protein energy (NPE) in the diet. Carbohydrate and fat are equally effective in promoting N retention (Forbes *et al.* 1939; Munro, 1964; Nakano & Ashida, 1975). The underlying mechanisms which are responsible for the change in N retention are not well understood but must involve changes in the relative rates of synthesis and breakdown of body protein. Changes in both protein synthesis and breakdown (Munro *et al.* 1959; Nakano *et al.* 1973) as well as changes in hepatic amino acid catabolic enzymes (Nakano & Ashida, 1969) have been implicated in the control of protein deposition by NPE.

In growing pigs (Reeds *et al.* 1980) and children (Golden *et al.* 1977) increases in N retention associated with increases in food intake are accompanied by higher rates of both protein synthesis and protein breakdown in the whole body. This paper reports the results of experiments to discover whether these changes in body protein turnover are the result of separate responses to dietary protein and to NPE, or whether they are invariably associated with alterations of N retention, howsoever produced.

METHODS
Animals and diets

The experiments were carried out with twelve female pigs which were the progeny of Large White boars and Large White × Landrace sows.

The basal diet consisted largely of cereals (Reeds et al. 1980) and when given at a rate of 90 g/kg body-weight $(W)^{0.75}$ per d (groups 30FB, 30CB and 30PB) supplied approximately 1·2 MJ metabolizable energy (ME) and 2·2 g of apparently-digestible N (ADN)/kg $W^{0.75}$ per d. Two diets of a higher energy:protein value were prepared by adding to 1 kg basal diet: 180 g lard (fat diet, group 30F), 407 g maize starch (one animal) or 264 g sucrose (three animals) (carbohydrate diet, group 30C). Additional vitamins, minerals and cellulose were also added.

A third diet (protein diet, group 30P) was also prepared by adding to 1 kg basal diet: 380 g soya-bean protein isolate (Promine D; Central Soya Co., Chicago, USA). The addition of the protein supplement had a negligible effect on the amino acid composition of the diet.

The diets were offered to the pigs once hourly at daily rates (g/kg $W^{0.75}$) of 124 (group 30S), 113 (group 30F) and 88 (group 30P), the aim being to achieve intakes of 1·8 MJ ME and 2·4 ADN/kg $W^{0.75}$ per d for groups 30F and 30C and 1·2 MJ ME and 4·3 g ADN/kg $W^{0.75}$ per d for group 30P. The intakes of ME and ADN that were attained are shown in Table 1.

Experimental design and procedures

Two PVC catheters (NT2; Portex Ltd, Hyde, UK) were introduced into the aorta of the pigs when the animals weighed between 17 and 20 kg (Fuller et al. 1977; Reeds et al. 1980). For 10 d after the operation the animals were offered the basal diet once hourly at a rate of 90 g/kg $W^{0.75}$ per d. When the animals weighed 22 kg they were randomly assigned to one of the three supplemented diets which were offered once hourly thereafter. When the

Table 1. *The body-weights (kg), apparent digestibilities of gross energy (GE) and nitrogen, the daily intakes of metabolizable energy (ME) and apparently digested N (ADN) of twelve female pigs offered basal diets† supplemented with fat (group 30F), carbohydrate (group 30C) or protein (group 30P) for 14 d and of the same pigs subsequently offered the basal diet for 14 d (groups 30FB, 30CB and 30PB)*

(Mean values ± 1 SEM)

| | | | | Apparent digestibilities | | | | Daily intake | | | |
| | | Body-wt (kg) | | GE (MJ/MJ) | | N (g N/g N) | | ME (MJ/kg $W^{0.75}$) | | ADN (g N/kg $W^{0.75}$) | |
Dietary group	n	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
30F	4	30·8	1·4	0·841	0·021	0·828	0·014	1·75	0·05	2·30	0·04
30FB	4	35·2	1·6	0·849	0·020	0·847	0·016	1·18	0·04**	2·17	0·05*
30C	4	29·6	0·9	0·851	0·025	0·835	0·008	1·58	0·08	2·31	0·06
30CB	3	35·3	1·3	0·861	0·012	0·868	0·016	1·16	0·03**	2·19	0·04*
30P	4	28·5	0·3	0·852	0·016	0·904	0·008	1·25	0·05	4·35	0·10
30PB	4	33·8	0·6	0·864	0·010	0·880	0·007	1·21	0·02	2·15	0·08**

$W^{0.75}$, body-weight$^{0.75}$ (metabolic body-weight).
Statistical significance of differences between supplemented and basal groups: * $P < 0.05$, ** $P < 0.01$.
† For details of diets and feeding schedule, see above.

animals reached 25 kg body-weight, bladder catheters were introduced and collections of urine and faeces were begun. At this time the amount of diet that was offered was adjusted to give the desired intakes of energy and protein for an animal weighing 28 kg.

The collections of excreta were continued for 7 d and during the last 4 d of this period 24 h measurements of gaseous exchange were carried out as described by Reeds *et al.* (1980). During the last day of the balance period an aqueous solution of L-[1-^{14}C]leucine (Radiochemical Centre, Amersham, Bucks) with sodium chloride (9 g/l) was infused for 6 h at a constant rate (13·5 μCi/h, 3·37 ml/h), a continuous record of the excretion of ^{14}C in expired air being obtained during the infusion. During the last 1·5 h of the infusion, when the specific radioactivity of the expired ^{14}CO$_2$ was constant, four blood samples (10 ml) were taken at 20 minute intervals. To these blood samples 1·25 μmol L-norleucine was added and the samples were stored at $-20°$ until analysed for [^{14}C]leucine. Body protein synthesis was calculated as the difference between the apparent irreversible loss of blood leucine and the rate of leucine catabolism (Reeds *et al.* 1980; Garlick *et al.* 1980) on the assumption that the relationship between the specific radioactivity of leucine in the blood and in the pool of leucine which acts as the precursor for protein synthesis was unaffected by the dietary supplements.

At the end of this period the bladder catheters were removed and the animals were offered the basal diet once hourly at a daily rate of 90 g/kg $W^{0·75}$ until they weighed 32 kg, when a second series of measurements of N balance (7 d), energy balance (4 d) and body protein turnover were made. During this period the animals were offered the basal diet at a rate appropriate to an animal weighing 34 kg.

The analytical measurements and calculations were made as described by Reeds *et al.* (1980), heat production being calculated with the equation given by Brouwer (1965). The statistical significance of differences between means was assessed by Student's *t* test for paired or non-paired groups as appropriate.

<div align="center">

RESULTS
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The apparent digestibilities of energy and N and the intakes of ME and ADN are shown in Table 1. With the exception of the high apparent digestibility of N in the high-protein diet there were no differences between diets in the digestibility of energy or of N. The amounts of each diet offered to the animals had been calculated to give equal protein intakes from the basal, carbohydrate and fat-supplemented diets and equal ME intakes from the protein-supplemented and basal diets. In the event there were small, but statistically significant, differences between the intakes of ADN/unit $W^{0·75}$ between groups 30C and 30CB and between groups 30F and 30FB.

N retention was higher, both in absolute terms and per kg $W^{0·75}$, during all the periods of supplemented feeding (Table 2). The rate of energy retention was also increased in groups 30C and 30F but not in the animals receiving the protein-supplemented diet. Although not reaching statistical significance the mean rate of fat deposition calculated from the difference between energy and N retention was lower in group 30P than in group 30PB. Increased intakes of carbohydrate and fat were associated with similar increases in daily heat production (Table 2) but the heat production of the animals that were given the high-protein diet increased by less than half. (30F−30FB = 129; 30C−30CB = 104; 30P−30PB = 43 kJ/kg $W^{0·75}$ per d).

The estimates of body protein turnover and leucine catabolism are shown in Table 3. Leucine catabolism (expressed as the proportion of the dose of ^{14}C excreted as CO$_2$) was decreased by 58% in groups 30C and 30F and increased by 73% in group 30P. In groups 30C and 30F body protein synthesis, expressed per kg $W^{0·75}$, was significantly greater than the values for groups 30CB and 30FB but the differences were small. There was a much

Table 2. *The daily heat production, retention of energy and nitrogen, together with an estimate of the daily retention of fat†, of twelve female pigs offered basal diets‡ supplemented with fat (group 30F), carbohydrate (group 30C) or protein (group 30P) for 14 d and of the same pigs subsequently offered the basal diet for 14 d (groups 30FB, 30CB and 30PB)*

(Mean values ± 1 SEM)

| Dietary group | n | Daily heat production | | Daily energy retention | | | | Daily N retention | | | | Daily fat retention (g fat/kg $W^{0.75}$) | |
| | | | | MJ | | kJ/kg $W^{0.75}$ | | g N | | g N/kg $W^{0.75}$ | | | |
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
30F	4	856	25	11·8	0·6	896	42	20·8	0·9	1·60	0·08	16·6	0·8
30FB	4	727	10**	6·9	0·8**	450	42**	13·6	0·7**	0·91	0·04**	7·9	1·0**
30C	4	876	20	9·2	1·2	746	57	18·2	1·2*	1·41	0·11	13·5	2·2
30CB	3	772	8**§	5·3	0·8*	387	37*	14·1	.1·0	0·92	0·03*	6·3	0·8*§
30P	4	785	15	5·7	0·8	466	58	16·6	0·8	1·36	0·10	7·0	1·4
30PB	4	742	16*	6·6	0·3	471	16	13·2	0·4*	0·94	0·02*	8·2	0·4

$W^{0.75}$, body-weight$^{0.75}$ (metabolic body-weight).

Statistical significance of differences between supplemented and basal groups (paired *t* test): * $P < 0.05$, ** $P < 0.01$.

† Calculated on the basis that the retention of 1 g N represents the retention of 148 kJ energy and 1 g fat 39·6 kJ energy.

‡ For details for diets, see p. 540.

§ Paired *t* test for diet 30C *v.* diet 30CB, three animals only.

Table 3. *Calculated daily rates of total body protein synthesis, protein breakdown and the catabolism of leucine (expressed as the proportion of dose of [1-^{14}C]leucine excreted as CO_2) in twelve female pigs offered basal diets‡ supplemented with fat (group 30F), carbohydrate (group 30C) and protein (group 30P) and in the same pigs subsequently offered the basal diet for 14 d (groups 30FB, 30CB and 30PB)*

(Mean values ± 1 SEM)

| Dietary group | n | Daily protein synthesis (g N/kg $W^{0.75}$) | | Daily protein breakdown (g N/kg $W^{0.75}$) | | Leucine catabolism (proportion of dose excreted as CO_2) | |
		Mean	SE	Mean	SE	Mean	SE
30F	4	6·01	0·19	4·14	0·18	0·088	0·004
30FB	4	5·58	0·17*	4·60	0·21	0·149	0·006***
30C	4	6·12	0·16	4·39	0·14	0·086	0·008
30CB	3	5·36	0·10*§	4·42	0·17	0·161	0·004***§
30P	4	7·40	0·16†	5·65	0·22†	0·259	0·012†
30PB	4	5·46	0·12**	4·43	0·20**	0·154	0·008***

$W^{0.75}$, body-weight$^{0.75}$ (metabolic body-weight).

Statistical significance of differences between supplemented and basal groups (paired *t* test): * $P < 0.50$; ** $P < 0.01$; *** $P < 0.001$.

† Statistical significance of difference from values of dietary groups 30C and 30F (unpaired *t* test): $P < 0.01$.

‡ For details of diets, see p. 540.

§ Paired *t* test for three animals only.

greater increase in protein synthesis in the animals receiving the high-protein diet. Protein breakdown, calculated as the difference between the apparent flux of amino acid N and N intake, was decreased by 10% in group 30F, unchanged in group 30C and increased by some 30% in group 30P.

<div align="center">DISCUSSION</div>

<div align="center">*Dietary energy, protein and protein turnover*</div>

In the present experiments, supplementation of the diet with either carbohydrate or fat increased daily N retention by 1·18 g N for each MJ increase in daily ME intake (1·21 g N/MJ with fat and 1·14 g N/MJ with carbohydrate). These estimates of the protein-sparing effects of dietary NPE are similar to those calculated for growing rats by Munro (1964) from the results of Forbes *et al.* (1939). In pigs, Fuller & Crofts (1977) obtained somewhat lower values than the present estimates, but their measurements were made in castrated male animals in which the rate of N retention at any given level of protein and energy intake was lower than that obtained in our experiment.

An increase in the protein intake of the animals was also associated with an increased rate of N retention. The measurements of protein synthesis described in the present paper were made to ascertain whether these increments in N retention, brought about by different changes in the diet, shared a common mechanism. The estimates of protein synthesis made from the specific radioacitivity of free leucine in the blood suggest that they do not.

Estimates derived in this way are based on the assumption, pointed out above, that the specific radioactivity (SR) of leucine incorporated into protein is the same as that of leucine in the blood. It is not yet established that this is so. Golden & Waterlow (1977) have pointed out that when the fraction of the flux of the tracer amino acid that is catabolized is known, then on the assumption (common to all methods of calculation of total body protein synthesis from a single amino acid) that the metabolism of the tracer is representative of the metabolism of all free amino acids, the flux of amino acid N can be calculated from the expression:

$$\text{Flux} = \frac{\text{rate of nitrogen excretion in the urine}}{\text{fraction of tracer catabolized}}.$$

This approach involves no assumption about the relative SRs of leucine in blood and that used for protein synthesis. Values for body protein synthesis (g N/kg $W^{0.75}$ per d) calculated in this way were 7·25, 9·56, 8·55 and 6·84 for groups 30F, 30C, 30P and pooled values for the basal diet respectively.

However not all urinary N is derived directly from the catabolism of amino acids by the animal. Especially with diets based on natural ingredients there may be considerable quantities of non-amino N derived from the diet which add to urinary N but not to urea and ammonia, the major end-products of amino acid catabolism. On this ground the excretion of urea and ammonia may be a better reflection of amino acid metabolism than total urinary nitrogen in animals receiving such diets as those used in the present and previous experiments (Reeds *et al.* 1980). The estimates of body protein synthesis (g N/kg $W^{0.75}$ per d) based on urinary urea and ammonia are 6·14, 6·42, 7·52 and 5·53 for groups 30F, 30C, 30P and pooled basal values compared with the values 6·01, 6·12, 7·40 and 5·47 derived from the SR of blood leucine.

It is inevitable that animals grow during an experiment of this nature and it had been planned to minimize the confounding effects of differences in body-weight by making measurements at three body-weights, giving supplements both before and after the animals were given the basal diet. Unfortunately it was found that at the heaviest weight (approximately 40 kg) the animals would not maintain a constant intake of supplemented

diet. Variations in food intake, particularly during the infusion of [^{14}C]leucine, invalidate the measurements of body protein synthesis and the necessary extrapolation of these measurements to 24 h periods (Reeds *et al.* 1980).

In calculating the amount of each diet to offer to the animals the amount per kg $W^{0.75}$ was kept constant and for this reason the majority of the results are expressed in these units. Our conclusions concerning the relative effects of the supplements upon the rates of energy and N retention are unaffected by their expression either per kg $W^{0.75}$ or as absolute quantities. However, when body protein synthesis and breakdown calculated from the labelling of blood leucine are expressed as absolute quantities, supplementation of the diet with NPE apparently has no effect upon the rate of protein synthesis and it follows that expressing the results in this way emphasizes the contribution of reduced protein breakdown to the change in N retention. Conversely, the higher the exponent of body-weight chosen to calculate the denominator, the greater becomes the apparent significance of changes in protein synthesis. It does however seem reasonable to conclude that the changes in N retention which were associated with changes in protein intake involved greater increases in the rate of protein synthesis than those associated with changes in the intake of carbohydrate or fat and that the changes in protein metabolism observed in our earlier measurements (Reeds *et al.* 1980) represented the end result of different and independent responses to alterations of energy and protein intakes.

The results shown in Table 4, where some of our previous (Reeds *et al.* 1980) and present measurements are compared, suggest that whilst the changes in protein synthesis in response to changing protein intake are the most marked, energy intake can also influence protein synthesis. It can be calculated that in animals of 30 kg body-weight, body protein synthesis increased by 0.88 g N for each g increase in ADN at constant energy intake and by 0.93 g N for each MJ increase in ME at constant intake of ADN.

Protein synthesis, protein deposition and heat production

It has been recognized for some years that many of the estimates of the energy costs of fat and protein deposition are heavily dependant on assumptions regarding the relationship between the maintenance component of heat production and body-weight (Thorbek, 1970; Kielanowski, 1976; Millward *et al.* 1976; McCracken *et al.* 1980). It has also been realized that the correlation between the rates of protein and fat deposition is usually so high that

Table 4. *Daily body protein synthesis, the catabolism of leucine, and the intakes of metabolizable energy (ME) and apparently digested nitrogen (ADN) in groups† 30P, 30PB and 30C in the present experiments and in dietary group 30H of Reeds* et al. *(1980).*

(The difference in protein synthesis between the dietary groups can be described by the equation $0.88x + 0.93y = z\ 0.02$ residual SD, where x is the change in ADN (g N/d), y the change in intake of ME (MJ/d) and z is the change in protein synthesis (g N/d))

Dietary group	Daily intake		Daily protein synthesis (g N/kg $W^{0.75}$)	Leucine catabolism (μCi/mCi infused)
	ME (MJ/kg $W^{0.75}$)	ADN (g N/kg $W^{0.75}$)		
30P	1.25	4.35	7.40	259
30PB	1.21	2.15	5.46	154
30F	1.75	2.30	6.07	87
30H	1.57	2.90	6.46	118

† For details of diets, see p. 540.

the precise separation of their energy costs is difficult (Pullar & Webster, 1977). In the present experiments the amounts of carbohydrate, fat and protein that were added to the basal diet were calculated to give equal increments in N retention with the aim of separating protein and fat deposition. In addition it was hoped that by basing calculations upon the differences between groups of animals of similar body-weights a necessary assumption as to the relationship between body-weight and maintenance heat production could be avoided (Pullar & Webster, 1977).

The comparison of the results of groups 30C and 30P (Table 2) in which the rates of protein deposition are nearly identical, suggests that the increase in heat production associated with the increase in fat deposition, presumably synthesized from carbohydrate, was 14 KJ/g. This is greater than the value that can be derived from the stoichiometry of triglyceride synthesis from glucose (Blaxter, 1967; Millward *et al.* 1976) but close to the values estimated in sheep for the efficiency of utilization for fattening of an intra-cardial infusion of glucose (Armstrong *et al.* 1960).

A similar calculation of the energetic efficiency of protein deposition, using the results of groups 30P and 30PB, suggests a heat increment of 17·1 KJ/g protein deposited but this calculation is less satisfactory owing to the slightly different rates of fat deposition in these two groups. If these differences are taken into account (assuming a heat increment of 14 KJ/g fat deposited) the apparent energy cost of protein deposition rises to 23·5 KJ/g protein deposited.

The suggestion has been made that the apparently high energy cost of protein deposition (expressed as the increase in heat production per unit protein deposited) is due, in part at least, to the energy required for body protein turnover. It has however been demonstrated in both pigs (Reeds *et al.* 1980) and children (Golden *et al.* 1977) that a significant proportion of body protein synthesis continues at N equilibrium. It follows that it is only the additional protein synthesis associated with growth that should be included in the heat increment which is statistically related to protein deposition.

The energy exchanges of the animals that were given the supplements of NPE were dominated by differences in the rate of deposition of body fat and the changes in protein synthesis were small. The calculation of the proportion of heat production associated with protein synthesis is extremely dependant on the accuracy of the estimate of the energy cost of fat deposition, which may not be the same in groups 30C and 30F. On the other hand, on all bases of calculation, there was a substantial change in body protein synthesis in the animals that were given the protein supplement and the differences in heat production and protein synthesis between groups 30P and 30PB suggest a heat increment of 3·8 KJ/g protein synthesized and 5·32 KJ/g if the energy cost of fat deposition is taken into account. These values are closer to the theoretical energy requirement for protein synthesis (Buttery & Boorman, 1976; Millward *et al.* 1976). They imply that, in the animals receiving the protein supplement a high proportion of the energy cost of the additional protein deposition can be ascribed to the additional protein synthesis associated with this treatment.

It is impossible to say whether this last conclusion, based on results obtained under a specific dietary circumstance, is applicable to animals given other diets. The results suggest that the relationship between protein synthesis and protein deposition is not fixed but is influenced by the nature of the diet. Thus if the change in protein deposition were the prin-ciple influence upon the energy cost of protein deposition, then the energetic efficiency of protein deposition would be variable. Further examination of the validity of this interpre-tation of the energy cost of protein deposition must await information on the extent to which other processes, which contribute to the heat production of the animal and which may be linked directly or indirectly to protein synthesis, vary with the nature of the diet.

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