

NUTRITION AND WHOLE-BODY PROTEIN TURNOVER IN THE CHICKEN IN RELATION TO MAMMALIAN SPECIES

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INTRODUCTION

Since the first indication by Schoenheimer (1942) of the dynamic state of body protein, it is well established that intracellular proteins are subject to substantial synthesis and degradation processes. As a result, accretion of protein in the whole body of animals is the reflection of small differences between synthesis and degradation rates. Because protein accretion, hence overall growth at early stages or egg, wool and milk production at maturity, is one of the major concerns in livestock husbandry as well as for human nutrition, there has been increasing interest in the regulation of these processes in recent years.

Investigation into the influence of nutrients on protein synthesis and degradation rates, expressed as either fractional rates (%/d) or absolute rates (g/d or g/kg body-weight^{0.75} per d) in the present review, in the whole body of animals has been greatly facilitated by the establishment of techniques for measuring synthesis rates *in vivo* such as continuous infusion (Waterlow *et al.* 1978) and large dose injection (McNurlan *et al.* 1979; Garlick *et al.* 1980*b*). Currently, estimates of protein degradation still depend mostly on indirect values deduced by subtracting growth rates from synthesis rates of body protein, due primarily to lack of adequate methods. Thus, any speculations relating to protein degradation in the present review are derived from such indirect measurements. Details of problems associated with the measurement of protein synthesis and degradation rates are reviewed elsewhere (Waterlow *et al.* 1978; Zak *et al.* 1979).

In the present review, the term 'protein turnover' is frequently used. However, its meaning is not always clear to everyone and, as Schimke (1970) pointed out, there has been confusion in the use of the term. In the following discussion, the term 'protein turnover'

Table 1. Comparison of the rate of whole-body protein synthesis in adult mammalian and avian species

Species	Body-wt (kg)	Whole-body protein synthesis (g/kg body-wt ^{0.75} per d)	Reference
Mammals			
Mouse	0.03	18.6	Muramatsu <i>et al.</i> (1985a)
Rat	0.51	17.4	Waterlow <i>et al.</i> (1978)
Rabbit	3.6	15.0	Reeds & Lobley (1980)
	3.6	19.0	Waterlow <i>et al.</i> (1978)
Goat	38	16.4	Muramatsu <i>et al.</i> (1988a)
	30–40	12.0–26.2	Muramatsu <i>et al.</i> (1989)
Sheep	40	15.9	Waterlow <i>et al.</i> (1978)
	63	15.7	Reeds & Lobley (1980)
Man	62	12.5	Reeds & Lobley (1980)
	77	16.7	Waterlow <i>et al.</i> (1978)
Cattle	500	16.1	Reeds & Lobley (1980)
	628	16.1	Lobley <i>et al.</i> (1980)
Birds			
Chicken	1.38	29.2	Muramatsu <i>et al.</i> (1987b)
	1.55	31.2	Muramatsu <i>et al.</i> (1987c)
	1.55	30.3	Hiramoto <i>et al.</i> (1989)

does not refer exclusively to the degradation process, as used by some authors, but generally to both synthesis and degradation processes, which are independently, though harmoniously, controlled in response to shifts of nutritional and physiological conditions. Only in a steady state, which is a special and limited instance of the more general condition, are the rates of the two processes the same.

The aim of the present paper is to investigate whether birds and mammals share the same underlying regulatory mechanisms in response to alterations in nutritional status. For this purpose, comparisons of avian species (i.e. chickens) with mammalian species are made in the area of whole-body turnover in relation to nutrient intake. In the well-fed state, when expressed as g/kg body-weight^{0.75} per d, the rates of whole-body protein synthesis are considerably higher in avian compared with mammalian species (Table 1), and probably so are the protein degradation rates. One of the reasons for this high rate of protein synthesis in chickens could be attributable to high body temperature, 41.7° compared with 37.0° for rats and humans, a difference that might account for about a 40% increase in protein synthesis (Laurent *et al.* 1978). Alternatively, some of the differences may also be ascribable to the different methods employed. Therefore, no direct comparison is made merely by comparing the absolute turnover rate between species but by looking at the responsiveness to changes in nutrient intake.

For the sake of convenience, separate considerations will be made of chronic effects such as results from the same dietary regimen for several days, and the acute, transient impact of dietary nutrient intake observed from day to day or within a single day. These time scales were chosen by assuming that a new steady state of whole-body protein turnover rates would be obtained within a few days of a change in diet. This was based on the fact that in chickens basal metabolism is attained after only 24 h of fasting in young chicks and a few days at most are necessary in adult fowls (Freeman, 1971), and that there appears to be a close relationship between whole-body protein turnover rates and energy metabolism (Waterlow *et al.* 1978; Webster, 1981, 1983; Muramatsu & Okumura, 1985).

Whether an experimental period is long enough to be classified as a chronic effect would

depend, in the first place, on species, age and body size of the animals to be examined. In humans, for example, a 3-week trial may not be long enough to establish reasonably constant rates of protein turnover in the whole body by changing dietary amino acid levels, as discussed by Young *et al.* (1987). On the other hand, a 1-week experiment may be quite long enough for attaining relatively constant rates of protein turnover in laying hens (Muramatsu *et al.* 1987*b, c*). In addition, the habits of food consumption vary from one species to another. In chickens, especially when they are young, the food is consumed throughout the day, whereas in humans nutrients are normally consumed as discrete meals. It follows, therefore, that for chickens on the one hand, a pseudo-steady state of whole-body protein turnover is attained as a consequence of an almost continual supply of nutrients. For humans, on the other hand, it is attained after repeated cycles of post-absorptive and feeding states. Thus, it should be born in mind that the definition of chronic or acute in the present review is provisional.

CHRONIC EFFECTS

PROTEIN SYNTHESIS AND REQUIREMENTS FOR PROTEIN AND/OR ENERGY

This section is devoted to elucidating the effects on whole-body protein synthesis of varying dietary protein or energy intakes, with particular emphasis on the requirements for these components.

As far as growing animals are concerned, whether avian or mammalian species, it would be fair to state that the requirement for protein is generally recognized as the level at which maximal growth and protein deposition are obtained. In this regard, the results in chickens imply that growth and whole-body protein kinetic measurements are closely related. Protein synthesis in the whole body of this species increased with increasing dietary protein intake up to an adequate level, and at above the optimum level no further increases were observed (Muramatsu *et al.* 1987*d*). Muramatsu *et al.* (1988*b*) examined in more detail the relationships between dietary protein-intake and growth, protein retention or whole-body synthesis in young chicks. The results are shown in Fig. 1, where the responses to altered protein intakes are expressed as a non-linear mathematical equation described by Mercer (1982). As shown clearly, the responses of each of these variables exhibited similar sigmoidal curves, the 90% asymptotic maximum values of which were obtained at 2.7, 3.0 and 2.9 g protein intake/d for body-weight gain, protein retention, and whole-body protein synthesis respectively. These values all confirm the recommended requirement of daily protein intake for optimal growth of 3.0 g, for birds of a similar body-weight (Scott *et al.* 1982).

In mammalian species, a comparable result has been reported. In rats protein synthesis in the whole body was increased with diets containing up to 200 g protein/kg, above which no further increase was observed and the synthesis rate remained constant (Laurent *et al.* 1984). Although no detailed quantitative estimation was made, yet the dietary level giving maximal protein synthesis would be in line with the dietary protein level of 150–200 g/kg, as recommended by National Research Council (1978).

In an essentially similar way to dietary protein intake, metabolizable energy (ME) intake may affect whole-body protein synthesis. Kita *et al.* (1989*b*) found in young chicks given a constant protein intake that when ME intake was deficient, protein synthesis rates decreased almost proportionally, whereas when ME intake was more than adequate, they remained constant. Fig. 2 shows the relationships between ME intake and body-weight gain, protein retained or whole-body protein synthesis with predicted curves from the non-

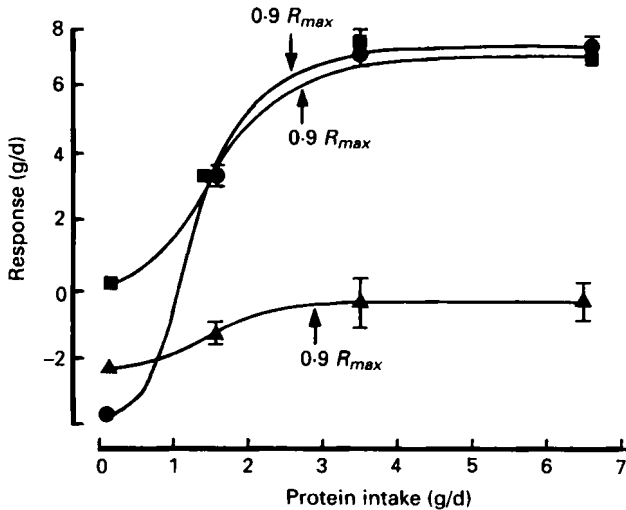


Fig. 1. Response curves in young chicks of body-weight gain (●), protein retention (▲) and whole-body protein synthesis (■) v. protein intake obtained by a four-parameter mathematical model (Mercer, 1982):

$$R = (b(K_{0.5})^n + R_{max}(PI)^n) / ((K_{0.5})^n + (PI)^n),$$

where R , PI , R_{max} , n and $K_{0.5}$ are response (g/d), protein intake (g/d), asymptotic maximum response, apparent kinetic order, and the intake for $0.5(R_{max} + b)$ respectively; b is the intercept on the response axis. Each response was well described by the regression equation with high R^2 , 0.98, 0.98 and 0.87 for body-weight gain, protein retention and whole-body protein synthesis respectively. Points are means with their standard errors represented by vertical bars. (Taken from Muramatsu *et al.* 1988*b*).

linear regression equations described by Mercer (1982). The 90% asymptotic maximum values for each variable were obtained at 155, 134 and 146 kJ/d for body-weight gain, protein retained and whole-body protein synthesis respectively, indicating that the value for whole-body protein synthesis fell between the other two variables that have usually been used as criteria of dietary energy requirements for growing birds. It should also be noted that the energy intake obtained at 90% asymptotic maximum values was close to, though slightly lower than, the recommended requirement of 156 kJ/d (National Research Council, 1984) and 163 kJ/d (Scott *et al.* 1982) for chickens of similar age and weight.

These findings from chickens are not definitive, but they suggest that there might be a close link between dietary energy requirement and whole-body protein synthesis, at least in this avian species. As for mammalian species, it has yet to be established whether a similar relationship exists, since no study has examined the effect of changes in ME intake with adequate supply of protein in sufficient detail to allow a quantitative analysis of whole-body protein synthesis and dietary energy requirement. The only studies which have been reported tend to involve a single level of dietary restriction (e.g. in obese human subjects, Garlick *et al.* 1980*a*). It is not known how far birds differ from mammals in response to ME intake *per se*.

As can be seen in the foregoing sections, altered protein and ME intakes brought about analogous responses of whole-body protein synthesis in the chicken. The question then arises whether these responses are caused through the same mechanism. Reeds & Fuller (1983) suggested from their findings in pigs (Reeds *et al.* 1981) that the effects of dietary protein and energy on protein metabolism are additive. This will suggest that they are probably brought about by different mechanisms linked to whole-body protein turnover.

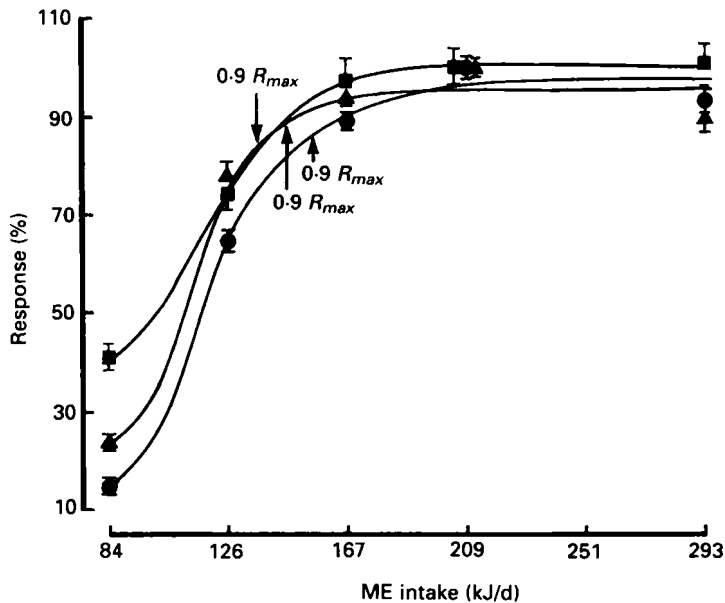


Fig. 2. Response curves in young chicks of body-weight gain (●), protein retention (▲) and whole-body protein synthesis (■) v. dietary metabolizable energy (ME) intake obtained by a four-parameter mathematical model (Mercer, 1982):

$$R = (b(K_{0.5})^n + R_{max}(PI)^n) / (K_{0.5})^n + (PI)^n,$$

where R , PI , R_{max} , n and $K_{0.5}$ are response (g/d), protein intake (g/d), asymptotic maximum response, apparent kinetic order, and the intake for $0.5(R_{max} + b)$ respectively; b is the intercept on the response axis. Points are means with their standard errors represented by vertical bars.

In attempting to provide evidence in chickens, a detailed factorial experiment was conducted to clarify the effects on whole-body protein synthesis of protein and ME intakes, both at deficient, adequate and excess levels (K. Kita, T. Muramatsu & J. Okumura, unpublished results). The values are presented as a three-dimensional response surface to illustrate the complicated response in a visual and comprehensible form (Fig. 3).

Some conclusions can be drawn from the response shown in Fig. 3. First, whole-body protein synthesis may be substantially influenced by the levels of both protein and ME intakes. The effect of increasing ME intake was smaller at deficient protein intake than at adequate or excess protein intake. Similarly, the effect of increasing protein intake was more obvious at adequate or excess ME intake than at deficient ME intake. Therefore, because of these separate influences of protein and ME intakes it is of primary importance to clarify at which level the starting point is set, and to describe the magnitude of the change in order to examine the response to alterations in these dietary components. Second, in the deficient region for both protein and energy, the effect on whole-body synthesis of increasing intake by 1 kJ protein appeared to be larger than that of increasing intake by 1 kJ total ME. When the scales for protein and total ME intakes in Fig. 3 are adjusted to be the same, the slope is clearly much steeper towards changes in protein intake. Third, the calculated maximum rates of whole-body protein synthesis were obtained at 154 and 155% of the requirement levels of dietary protein and ME intakes respectively according to the values recommended by Scott *et al.* (1982). At the recommended requirement levels (52 kJ, i.e. 3 g/d for protein and 167 kJ/d for ME), however, the value for whole-body protein

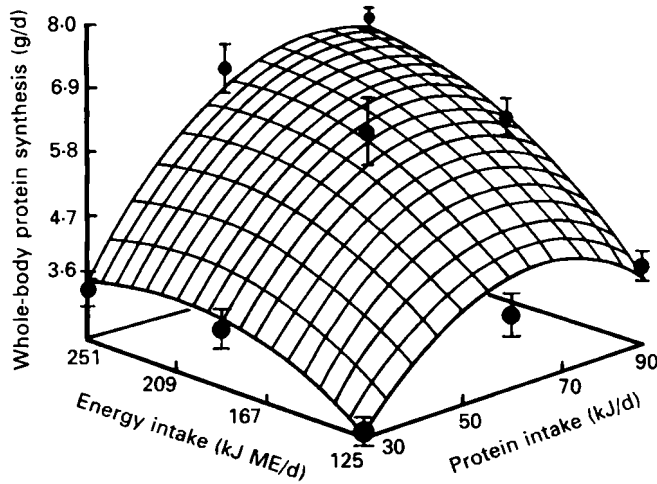


Fig. 3. Response surface of whole-body protein synthesis in chicks fed at varying dietary protein and total energy intakes. Values are means with their standard errors represented by vertical bars. ME, metabolizable energy. (Taken from K. Kita, T. Muramatsu & J. Okumura, unpublished results).

synthesis had already reached 87% of the maximum. The fact that essentially similar responses of body-weight gain and protein retained were found in the same experiment suggests again the possible usefulness of whole-body protein synthesis as an index of dietary protein and ME requirements in growing chickens.

The conclusion emerging from the previously mentioned experiment on chickens might not be directly applicable to other animal species. In these species existing evidence is more conflicting and complex so that it is more difficult to draw firm conclusions. For example, little effect of energy intake on whole-body protein kinetics has been reported in humans (Garlick *et al.* 1980*a*; Winterer *et al.* 1980), whereas Hoffer *et al.* (1984) found a reduction in whole-body protein synthesis of obese human subjects when energy intake was severely restricted.

To illustrate the relationship between whole-body protein synthesis and protein or ME intake more clearly, in the search for a complete picture of controls of whole-body protein synthesis by these dietary components, various literature sources covering several species were examined, and the relationship between whole-body protein synthesis, protein intake and ME intake was analysed with respect to their requirements. Fig. 4 shows the levels of dietary protein and ME intakes at which whole-body protein synthesis was measured in twenty-two studies available in the literature. The scatter or the measurement points in Fig. 4 suggests that speculation on response trends should only be made by pooling the results from a whole class. No single class consisting of several studies could cover the requirement region over a sufficiently wide range except for chickens.

Multiple regression of protein synthesis *v.* intakes of dietary protein and ME was calculated and only the terms significant at $P < 0.05$ were included in the corresponding models. The resultant regression equations for chickens, human subjects, non-human mammalian species including rats, pigs and cattle, and pooled classes were as follows:

(1) chickens

$$PS = 14.05 + 0.2025 PI - 0.000637 PI^2, \\ (R^2 0.58, P < 0.001, \text{d.f. } 41),$$

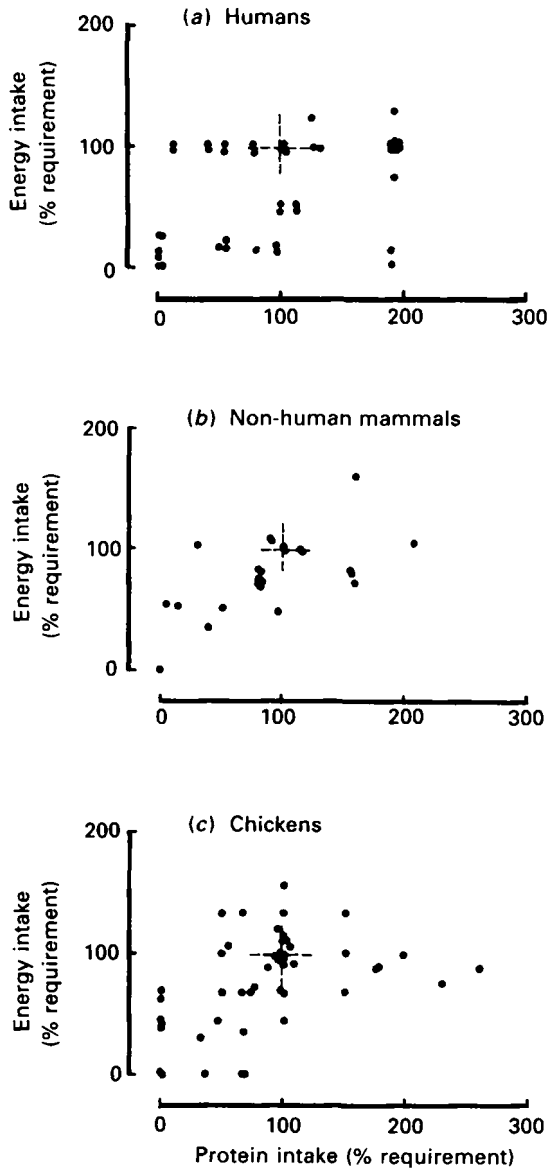


Fig. 4. Distribution of measurement points of dietary protein and energy intakes at which whole-body protein synthesis was determined in the literature. The values were taken from twenty-two studies, covering human subjects, non-human mammalian species and chickens, and were expressed with respect to percentage of requirements for both protein and energy intakes. The sources were: Conway *et al.* (1980), Garlick *et al.* (1980*a*), Gersovitz *et al.* (1980), Reeds *et al.* (1980), Winterer *et al.* (1980), Motil *et al.* (1981*a, b*), Reeds *et al.* (1981), Adibi *et al.* (1982), Hoffer *et al.* (1984), Laurent *et al.* (1984), Muramatsu *et al.* (1986), Yang *et al.* (1986), Fuller *et al.* (1987), Lobley *et al.* (1987), Muramatsu *et al.* (1987*a, b, c, d*), Kita *et al.* (1989*a, b*), and K. Kita, T. Muramatsu & J. Okumura (unpublished results). Where necessary, conversion of amino acid flux to protein flux was done according to the amino acid content in the whole body (Smith, 1980).

(2) human subjects

$$PS = 6.50 + 0.0194 PI - 0.000063 PI^2 + 0.2665 MEI, \\ (R^2 0.333, P < 0.001, \text{d.f. } 33),$$

(3) non-human mammalian species (rats, pigs and cattle)

$$PS = 8.99 + 0.2025 PI - 0.000637 PI^2, \\ (R^2 0.421, P < 0.001, \text{d.f. } 19),$$

(4) pooled (1, 2 and 3 included).

$$PS = 10.98 + 0.0612 PI - 0.000214 PI^2 + 0.07481 MEI, \\ (R^2 0.178, P < 0.001, \text{d.f. } 99),$$

where *PS*, *PI* and *MEI* are whole-body protein synthesis (g/kg body-weight^{0.75}/d), dietary protein intake (% requirement) and dietary ME intake (% requirement).

A quadratic response of whole-body protein synthesis to dietary protein intake indicates that there is an optimal protein intake to give maximum values ranging from 130 to 159% of the dietary protein requirements for individual classes. However, the response has already reached 93–98% of the corresponding maximum values at the recommended requirement level of dietary protein intake. In contrast, the effect of ME intake in individual classes is not significant, except for humans. These results, indicating that the effect of ME in birds and non-human mammals failed to reach statistical significance, were unexpected. Inter-experimental variations may have offset the ME effect. In addition, the results for chickens and non-human mammalian species were obtained primarily from experiments where animals were growing, whilst those for humans were derived mostly from experiments at adulthood. The difference in age might be partly responsible for the differences in responses.

In the pooled classes, there are quadratic and linear effects of protein intake and ME intake respectively, with relatively poor fit (R^2 0.178) which is ascribed primarily to inter-class variations. Because of this large variation, a careful interpretation should be given to the regression equation for the pooled classes. Even the definition of 'requirements' would be different between human subjects and growing animals. So-called 'allowance' frequently used in human studies, which is considered here in the present review as 'requirement', includes a safety margin, say 30%, on top of the minimal needs of dietary protein intake. By contrast 'requirements' in studies with farm livestock and laboratory animals may correspond to the minimal value that would produce maximal growth and protein retention. Thus, the regression equations are considered approximate and provisional. Nevertheless, it does appear that whole-body protein synthesis in avian and non-human mammalian species responds in a similar way to these two major dietary components. The fact that there appear to be no considerable deviations of the results in chickens from those in non-human mammalian species suggests that the regulatory mechanisms of whole-body protein synthesis in response to dietary changes in protein and/or ME are the same in avian and non-human mammalian species, although there is no *a priori* reason why this should be so. The way in which protein synthesis in various animal species is related to the requirements of these dietary components must await further investigation.

AMINO ACID DEFICIENCY

Does the level at which maximal rates of protein turnover, particularly synthesis, are attained have any relevance to requirements for individual amino acids? Limited evidence

from experiments in mammalian species suggests that the response of whole-body protein turnover to dietary amino acids is apparently the same as that to dietary protein. By changing dietary leucine from deficient to excess levels, Meredith *et al.* (1982) demonstrated that in humans whole-body protein synthesis was increased almost linearly to a breakpoint, probably representing the leucine intake required for maximum net protein accretion, from which the synthesis rate reached a constant value. These studies have been described in full and extended to include similar studies with valine and lysine (Meguid *et al.* 1986*a, b*; Meredith *et al.* 1986). A similar pattern of responses of whole-body protein synthesis to increasing protein intake was observed in rats (Laurent *et al.* 1984). In avian species, however, the answer to this question cannot be given because no comparable systematic studies are available. Therefore, the following description is restricted to qualitative changes in protein turnover when one or more essential amino acids are deficient in a diet. Another important question is the extent to which certain amino acids have a regulatory role in protein turnover in tissues such as liver and muscle *in vivo*, and thereby in the whole body. This concept is attractive to many scientists in the medical field. For example, Millward & Rivers (1988, 1989) have introduced the concept of the anabolic drive to describe this regulatory influence of amino acids, arguing that such influences of nutrients should be included in the criteria by which nutrient requirements are assessed. However, this subject is beyond the scope of the present review, and the readers are referred to recent papers (Chua *et al.* 1979; Garlick & Grant, 1988; Jepson *et al.* 1988*a*; Millward, 1989).

It is legitimate to state, at present, that in avian species deficiency of any single essential amino acid would lead to a reduced rate of protein synthesis and possibly protein degradation in the whole body. Supplementing with these amino acids would then restore the lowered turnover rates. Hiramoto *et al.* (1990) found, for example, that in laying hens fed on either maize-gluten meal or isolated soya-bean protein as the only source of dietary protein to produce deficiency in lysine or methionine respectively, both protein synthesis and degradation rates in the whole body showed a remarkable enhancement when these amino acids were supplemented. The findings of reduced whole-body protein synthesis in young chicks when deprived of dietary sulphur-containing amino acids or histidine (Kino & Okumura, 1987*a*) is also in line with the result in laying hens.

In mammalian species the effects of deficiency of a single essential amino acid are generally in good agreement. Conway *et al.* (1980) found in humans that supplementing a lysine-free diet with this amino acid substantially enhanced whole-body protein turnover rates. As already stated, leucine, valine or lysine deficiency brought about a reduction in whole-body protein synthesis in humans (Meredith *et al.* 1982, 1986; Meguid *et al.* 1986*a, b*) and in rats (Laurent *et al.* 1984). In goats, Muramatsu *et al.* (1989) also found that supplementing a urea diet with methionine that is resistant to degradation in the rumen resulted in enhanced turnover rates in the whole body.

In growing pigs, in contrast, Fuller *et al.* (1987) failed to observe any significant changes in the whole-body protein synthesis by supplementing a diet believed to be deficient in lysine with this amino acid. As far as the response to amino acid deficiency is concerned, this is the only exception. Although the exact reasons for the anomaly in this particular species remain to be investigated, it is possible that the extent of lysine deficiency in their study was just not large enough to bring about any detectable changes in the whole-body protein turnover in growing pigs.

AMINO ACID ADDITION TO A PROTEIN-FREE DIET

Where young chickens were subjected to protein starvation, i.e. were made deficient in all essential amino acids, supplementing a protein-free diet with methionine in combination

with arginine improved nitrogen balance (Muramatsu & Okumura, 1979*a, b*, 1980*a*). This enhanced whole-body protein synthesis, and to a lesser extent degradation (Muramatsu *et al.* 1986). The effect was also observed in the liver and the jejunal mucosa (Muramatsu *et al.* 1983), and to an even greater extent in skeletal muscle (Muramatsu *et al.* 1985*b*). Cystine and glutathione may bring about a similar response of enhanced whole-body protein synthesis in the presence of arginine in protein-starved chicks, as judged from the results of N balance studies (Muramatsu & Okumura, 1980*b*; Muramatsu *et al.* 1984). This phenomenon might be thought surprising, since there is no amino acid given in the diet except for methionine and arginine. However, the N-sparing effect of methionine alone or in combination with other amino acids has often been found in mammalian species, including dogs (Allison *et al.* 1947), pigs (Lubaszewska *et al.* 1973) and rats (Yoshida & Moritoki, 1974; Yokogoshi & Yoshida, 1979). Bearing in mind the fact that there is a large amount of amino acid flux due to whole-body protein degradation and re-synthesis even in the protein starvation state, improved N balance and enhanced whole-body protein turnover rates might be more understandable than it seems. In protein-starved animals, the amino acids released by proteolysis would appear to be deficient in methionine relative to the needs for synthesis. Biochemical evidence seems in favour of the possibility of relative deficiency of amino acid in protein-starved animals since methionine would probably be oxidized much faster than any other amino acids in rats and chickens (Aguilar *et al.* 1972; Neale & Waterlow, 1983; Kino & Okumura, 1987*b*). Bressani *et al.* (1965) also found larger values for urinary S:N ratios in protein starvation than in normally fed dogs, suggesting a relatively greater loss of S-containing amino acids compared with others during protein depletion. In animals, methionine and cystine are also important constituents of feather, fur and hair proteins, being as much as 10% in the case of cystine (Mitchell, 1959). The component amino acids of these proteins are likely to be lost from skin surface, hence not returnable to the free amino acid pool once they are synthesized, and this would in turn accentuate the needs for S-containing amino acids in protein starvation.

Whatever the primary reason for bringing about the N-sparing effect may be, all previously stated evidence supports the idea that the mitigation of deficiency of a certain essential amino acid, methionine in this particular case, would probably result in enhanced rates of whole-body protein turnover in chickens, even when they are subjected to protein starvation. The evidence so far obtained in mammalian species is circumstantial because no measurement on whole-body protein turnover rates has been attempted in conjunction with N balance studies. However, the enhanced whole-body protein turnover rates are very likely when a protein-free diet is supplemented with methionine, and the turnover responses between birds and mammals may well resemble each other in this respect.

ACUTE EFFECTS

Unlike chronic effects, the body of available evidence concerning acute effects on whole-body protein turnover are so limited in avian and mammalian species that any kind of formal comparisons are not possible at present. In the following section, therefore, much emphasis is placed on what is known in chickens together with pieces of relevant information in mammals.

FASTING AND REFEEDING A DIET OR SPECIFIC NUTRIENTS

Table 2 shows changes in whole-body protein synthesis in response to refeeding a single meal of a whole diet in chicks fasted for 48 h (Aoyagi *et al.* 1989). After the refeeding procedures, the whole-body protein synthesis rate, which was low as a result of fasting,

Table 2. Effect of refeeding a single meal of semi-purified diet on fractional and absolute rates of protein synthesis in the whole body of fasted chicks (Taken from Aoyagi *et al.* 1989)

(Mean values for six chicks)

Treatment	Fractional rate (%/d)	Absolute rate (g/kg body-wt per d)
Fed	34.0 ^a	55.4 ^a
Fasted (48 h)	23.4 ^d	41.7 ^d
Refed: 2 h	28.1 ^c	50.0 ^{bc}
6 h	31.1 ^b	54.3 ^{ab}
24 h	27.0 ^c	48.3 ^c
Pooled SEM (25 df)	1.0	1.7

a, b, c Means with unlike superscript letters within the same column were significantly different ($P < 0.05$).

significantly increased within 2 h, although it did not completely recover to the full-fed state in terms of either the fractional or absolute rate. After 6 h of refeeding, the absolute rate almost returned to the prefasting level whilst the fractional rate did not. Because the amount contained in the meal was small, approximately half the daily food consumption, these values declined again at 24 h after the refeeding procedure.

These results clearly indicate that whole-body protein synthesis can respond to dietary alterations within as short a period as 2 h under conditions of total fasting followed by refeeding of a whole diet. The enhanced protein synthesis was primarily explained by an increase in protein synthesis per unit RNA, but not in terms of RNA:protein ratio. In fasted chickens, in contrast, Muramatsu *et al.* (1987a) found that a reduced fractional rate of protein synthesis in the whole body was accounted for by reductions in both protein synthesis per unit RNA and RNA:protein ratio compared with the fed state. It would appear, therefore, that the metabolic consequences of fasting on protein turnover involve mechanisms which cannot immediately be reversed during refeeding. The fact that the amount of total RNA remained low, and became even lower towards 24 h after refeeding, suggests that steps at translation rather than transcription were modulated during the early refeeding phase, possibly by increasing the rate of initiation of translation, which was inhibited due to fasting (Harmon *et al.* 1984), although this does not necessarily rule out the possibility of changes in transcriptional rates of mRNA.

Refeeding mixed diets in post-absorptive human subjects has resulted in different and conflicting responses of whole-body protein synthesis depending, probably, on the protein level employed and route of nutrient administration. Hoffer *et al.* (1985) found in humans a small but significant increase in whole-body protein synthesis when an adequate amount of protein was fed. When an amino acid mixture was administered parenterally to humans, Pacy *et al.* (1988) found a somewhat larger increase, 30%, in whole-body protein synthesis compared with that of a fasted state. On the other hand, Melville *et al.* (1989) could not find any significant changes in whole-body protein synthesis in human subjects. Motil *et al.* (1981b) found in humans that at a very low level of protein intake there was a significant decrease in whole-body protein synthesis, whereas there was an increase at a marginally deficient level and no change at an excess level, the extent of alterations ranging from -15 to 15%. However, the decreased response found by Motil *et al.* (1981a) at the very low protein intake could be considered as an extreme example when the balance of protein and energy in the meal is distorted considerably, and thereby can be regarded principally as an effect of carbohydrate or fat intake rather than protein intake *per se*, as discussed later. The

reason for varied responses, from no change to an increase as large as 30%, is not clear. Therefore, the consequence of protein consumption after fasting on whole-body protein synthesis in mammalian species could tentatively be defined as being unchanged or increased.

The response of whole-body protein degradation to refeeding of an adequate diet in the fasting state is not available for avian species. The evidence from human studies indicates that there is a considerable reduction, by 31–65%, in whole-body protein degradation (Conway *et al.* 1980; Motil *et al.* 1981*b*; Hoffer *et al.* 1985; Melville *et al.* 1989). Melville *et al.* (1989), feeding adequate hourly meals, reached the conclusion that the acute regulation of whole-body protein kinetics, e.g. in response to meals, was primarily achieved by alterations in whole-body protein degradation. The inhibited degradation was also substantiated by parenteral administration of amino acids. When amino acids were infused intravenously, a reduction of about 20% in whole-body protein degradation was found (Pacy *et al.* 1988).

The reduced degradation after refeeding discussed previously is also observed in rat muscle *in vitro* (Li & Wassner, 1984). There is good reason to assume that certain hormones such as insulin are involved in altered whole-body protein degradation. Refeeding a diet enhances the circulating level of insulin, and the increased insulin, in turn, reduces the muscle protein degradation *in vitro* (Li & Wassner, 1984). Thus, the hormone appears to play an important regulatory role in protein degradation as well as protein synthesis in the whole body. A number of studies also suggest the involvement of other hormones, but the subject is beyond the scope of the present review. The readers are referred to recent papers for further reading (Rabouli & Martin, 1977; Millward *et al.* 1983, 1988; Garlick *et al.* 1985, 1988; Eisemann *et al.* 1986; Jepson *et al.* 1988*b*).

If a diet in which the balance of energy and protein is distorted is refed to fasted animals, the directions of the response of protein turnover rates are likely to be different. In chickens, our preliminary results indicated that the extent of changes in whole-body protein synthesis varied in response to meals consisting of carbohydrate, fat and protein (Y. Aoyagi, T. Muramatsu & J. Okumura, unpublished results). Likewise in mammals, Adibi *et al.* (1982) found unchanged total N flux with reduced amino acid oxidation by feeding exclusively carbohydrate to postabsorptive obese subjects, implying an increase in whole-body protein synthesis. Nair *et al.* (1988) also reported that in postabsorptive subjects the infusion of β -hydroxybutyrate resulted in elevated whole-body protein synthesis. These studies are relevant to the question of the relationship between diet-induced thermogenesis and protein turnover. However, further discussion will have to await the accumulation of a more concrete body of evidence.

PROTEIN DEPLETION AND REPLETION

Unlike the previously mentioned fasting–refeeding condition, where dietary energy is totally removed before the commencement of refeeding, the effect of nutrient depletion and repletion shows an entirely different response in the presence of an adequate energy intake throughout a treatment period.

In this category, the available information in avian species is exclusively limited to dietary protein consumption. During the course of protein depletion and repletion with an adequate energy supply, whole-body protein kinetics in laying hens were studied on a day-to-day basis. As might be expected, it was found that synthesis was decreased by feeding a protein-free diet. The rate of fall was rapid initially, but declined to almost a plateau basal level after 7 d of protein starvation (Muramatsu *et al.* 1987*c*; see Fig. 5). A similar pattern of response was also observed for the rate of protein degradation, although it was less

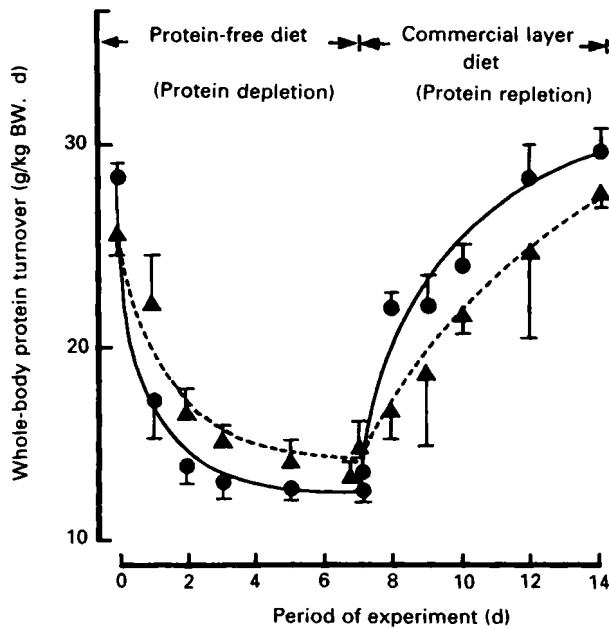


Fig. 5. Changes in whole-body protein turnover in laying hens fed on a protein-free (protein depletion) or commercial layer (Protein repletion) diet (●—●), synthesis; (▲—▲), degradation; BW, body-weight. Points are means with their standard errors represented by vertical bars. Mean values were significantly different from the corresponding initial values ($P < 0.05$). (Taken from Muramatsu *et al.* 1987c).

pronounced. When a protein-containing diet was refed, whole-body protein synthesis and degradation increased promptly and sharply, although the changes in degradation were less marked. The protein turnover rates increased more rapidly for first 1–2 d, followed by a diminished incremental rate, and finally at 7 d of refeeding approached steady plateau rates that were similar to those in the pretreatment period.

An extraordinary aspect related to this finding in laying hens was the fact that almost half the reduction in the whole-body protein synthesis by protein starvation was accounted for by a reduction in that of the liver and oviduct (Muramatsu *et al.* 1987c). The high sensitivity of liver and particularly the oviduct to the change in dietary protein supply is probably a specific property in this species, especially when they are at a laying stage. This is perhaps the consequence of long genetic selection for a high egg production rate and it is therefore not as surprising as it seems because both the liver and oviduct are the primary sites of egg yolk and albumen protein synthesis respectively. Obviously, the egg formation process involves the steroid-induced transcription of mRNA encoding egg proteins (Deeley *et al.* 1977; McKnight & Palmiter, 1979; Sanders & McKnight, 1985). However, the way in which alterations in nutrient supply interact with steroid-induced gene transcription is almost entirely unknown. Specific nutrients could affect the synthesis of DNA-binding regulatory proteins, their binding affinity, processing of premature mRNA or stabilization of mRNA.

In mammalian species, if there is a tissue comparable with the oviduct of laying hens, it would be the mammary gland of lactating animals. In rats, protein synthesis in mammary gland and liver was clearly influenced by dietary protein quality and feeding level, the changes being more remarkable in the mammary gland (Sampson & Jansen, 1984, 1985;

Table 3. Comparison of responses of whole-body protein turnover between avian and mammalian species in relation to nutritional status

Species		Avians	Mammals	
Chronic effect on synthesis	Protein	Deficient	Reduction	Reduction
		Excess	No change	No change
		Relevance to requirement	Possible	Possible
	Energy	Deficient	Reduction	No change (?)
		Excess	No change	No change
		Relevance to requirement	Possible	?
	Amino acid	Deficient	Reduction	Reduction
		Moderate excess	?	No change
		Relevance to requirement	?	Possible
Acute effect on synthesis and degradation	Refeeding after total fasting			
Protein repletion after depletion	Synthesis	Elevation	Elevation or no change	
	Degradation	?	Reduction	
	Synthesis	Elevation	?	
	Degradation	Elevation	?	

Sampson *et al.* 1986). After protein starvation for 1 d in lactating rats, Jansen & Hunsaker (1986) reported that the sum of the reductions in protein synthesis in mammary gland and liver was about 8 g/kg body-weight per d. This amount corresponds to about 26% of the whole-body protein synthesis in rats fed on an adequate diet (McNurlan & Garlick, 1980) and, therefore, probably accounts for a greater proportion of reduced whole-body protein synthesis during protein starvation.

OUTLOOK AND CONCLUSIONS

Whilst surveying the nutritional regulation of whole-body protein turnover in avian species in comparison with mammalian species it is clear that the current status of our understanding of the subject of protein turnover is far from complete. Table 3 summarizes what is known about whole-body protein turnover in a comparative form for avian and mammalian species. Although there is no *a priori* assumption that the control mechanisms are the same for the two species, the evidence presented hitherto has shown that the responses, and therefore underlying regulatory mechanisms, of whole-body protein turnover in avian species are not evidently different from those in mammalian species. This is true despite the fact that there is a clear contrast in the metabolism of some nutrients, such as the major nitrogenous end-product in urine, i.e. uric acid in birds and urea in mammals.

The literature on avian species shows that the acute consumption of a whole diet after total starvation or of a protein-containing diet after protein starvation leads to a rapid and prompt increase in whole-body protein synthesis. Chronic changes in protein and ME consumption from deficient to adequate levels also enhance whole-body protein synthesis, but there is no further increase at excess levels. This suggests that there may be a close quantitative relationship between whole-body protein synthesis and dietary protein and ME requirements. Unfortunately no information is available so far for other nutrients. In this regard, it is perhaps worth noting the statement of Young *et al.* (1987), who argued that more attention should be paid to protein kinetic studies in the whole body and to

understanding the underlying mechanisms before hastening to draw a conclusion on the relationship between nutrient requirements and whole-body protein turnover.

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