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Übersicht

Nutrient interactions with reference to amino acid and protein metabolism in non-ruminants; particular emphasis on protein-energy relations in man[1])

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Summary: Because the regulation of protein and energy balance is of major research interest in the nutrition and physiology of humans and animals, a selected account of interactions between protein and energy is given here, with particular emphasis on studies in human subjects. The discussion begins with reference to the relations between protein and energy intakes and nitrogen balance; selected aspects of the relations between protein dynamics and energy metabolism among the various mammalian species are then considered. This leads to a brief account of oxidative amino acid catabolism and its relevance to the assessment of amino acid requirements, particularly in adult man. It is concluded that obligatory oxidative losses of amino acids can be used to predict or approximate amino acid requirements in children and adults. The nitrogen-sparing properties of carbohydrate and lipid-derived fuels are then considered. Despite the well-known and profound, yet differential, impacts of dietary protein and energy sources, and their interactions on body protein balance, there remain wide gaps in our understanding of the mechanisms responsible for their effects, such as the quantitative and mechanistic involvement of hormones, including insulin and the counter-regulatory hormones, and the roles played by the major amino acids responsible for the interorgan transport of nitrogen and the regulation of urea production. Additional studies focusing on metabolic nitrogen trafficking would significantly enhance an understanding of how protein and energy interact to achieve the efficient utilization of dietary protein for maintenance and promotion of lean body gain.

Zusammenfassung: Da die Balance zwischen Proteinstoffwechsel und Energieumsatz von großer Bedeutung in der Ernährung und Stoffwechselphysiologie von Mensch und Tier ist, wird eine Gesamtbetrachtung der Interaktionen zwischen Protein und Energie vorgenommen mit spezieller Berücksichtigung von Studien am Menschen selbst. Zunächst wird auf die Beziehung zwischen Protein und Energieaufnahme zur Stickstoffbilanz eingegangen und danach auf ausgewählte Aspekte der Relation des Proteinstoffwechsels zum Energieumsatz bei verschiede-

[1]) Based on a talk presented at the 6th International Symposium on Protein Metabolism and Nutrition, June 9–14, 1991, Herning, Denmark

nen Säugetier-Spezies. Dies führt zu einer kurzen Besprechung des oxidativen Aminosäurenabbaus und seiner Bedeutung für die Bestimmung des Aminosäurenbedarfs vor allem beim Erwachsenen. Es wird der Schluß gezogen, daß die unausweichlichen Verluste durch Oxidation der Aminosäuren für die Berechnung oder zumindest Schätzung des Aminosäurenbedarfs von Kindern und Erwachsenen geeignet sind. Die N-sparenden Eigenschaften der Kohlenhydrate und Lipide werden danach besprochen. Trotz der gut bekannten und profunden, wenn auch vielschichtigen Wirkungen der Nahrungsproteine und Energieträger sowie ihrer Interaktionen auf die Balance der Körperproteine bleiben weite Lücken in unserem Verständnis der Wirkungsmechanismen übrig. Dazu gehören z. B. die quantitative und mechanistische Einwirkung der Hormone einschl. Insulin und der gegenregulatorisch wirksamen Hormone sowie die Rolle derjenigen Aminosäuren, die hauptsächlich für den Transport des Stickstoffs zwischen den Organen und für die Regulation der Harnstoffproduktion verantwortlich sind. Zusätzliche Studien mit dem Ziel, die Stoffwechselwege des Stickstoffs zu ergründen, würden das Verständnis der Frage verbessern, wie Proteine und Energie zusammenwirken, um eine effiziente Verwertung des Nahrungsproteins für die Erhaltung und Steigerung der fettfreien Körpermasse zu erreichen.

Key words: protein; energy; amino acids, turnover, synthesis, breakdown, oxidation; nitrogen balance; sparing; glucose; carbohydrate; lipid, requirements; hormones, insulin; counter-regulatory hormones; glucagon; epinephrine; glucocorticoids; muscle; basal metabolic rate

Schlüsselwörter: Proteine; Energie; Aminosäuren-Stoffwechsel; Synthese, Abbau, Oxidation; Stickstoff-Bilanz; Sparmechanismen; Glucose; Kohlenhydrate; Lipide, Bedarf; Hormone, Insulin; gegenregulatorisch wirksame Hormone; Adrenalin; Glucocorticoide; Muskeln; Grundumsatz

Introduction

The efficient use of dietary proteins (beginning with the liberation of their constituent amino acids during the course of digestion) for meeting maintenance and growth needs requires an adequate level of energy-yielding substrates and of all other essential nutrients, including the essential fatty acids, macro- and micro-mineral elements, and vitamins. Inadequate or excessive intakes of one or more of these dietary constituents lead to a reduction in the anabolic value of ingested protein. From this standpoint, alone, a comprehensive account of nutrient interactions with reference to protein and amino acid metabolism and nutrition demands a broad survey of experimental nutrition and of nutritional biochemistry. However, for the present purposes, it would seem useful to narrow the scope of our discussion, giving attention to interactions that are likely to be of major practical importance in the nutrition of non-ruminants. Since my research has been concerned largely with protein nutrition/metabolism in human subjects, I will focus most of the discussion on studies conducted in humans.

The regulation of protein and energy balance continues to be a major field of research interest in nutrition and physiology of humans and animals. Considerable emphasis in animal production is now given to enhancement of lean tissue growth, or protein accretion, together with a

less substantial deposition of body fat. Thus, there is good reason for a narrowing of the scope to considerations of protein-energy interactions. Further, the sensitivity of body nitrogen balance to both energy and protein intake has been recognized for years and many of the earlier, basic observations in this area were reviewed sometime ago by Munro (58), and a few years later by Calloway and Spector (14). These two important contributions gave a particular emphasis to the impact of dietary energy and protein on body nitrogen balance. The relationships between dietary protein and energy intake on growth in mammals (e.g., (15)) and in avian species (60) have also been reviewed recently. Hence, this review will attempt to identify some of the underlying metabolic mechanisms and processes that might be of importance as we explore the nutritional significance of protein and energy interactions. Included will be a brief overview of selected associations between protein and energy metabolism among various mammalian species. The objective is to try to establish that some of the relationships are broadly similar among these species and to determine whether they apply to human beings, the latter being the focus of this review. From this, we will also ascertain to what extent protein metabolism contributes to body-energy expenditure and metabolic rate. We then turn to the relationships between protein turnover, amino acid oxidation and utilization, with the purpose of assessing how energy status and the provision of specific energy-yielding substrates might affect amino acid (nitrogen) and thus the efficiency of N-utilization. We will also explore the effects of various energy-yielding substrates on whole body, muscle protein, and specific amino acid turnover. Again, the emphasis will be in man, particularly, because much of the work in this area has been devoted to clinical studies, with the purpose of improving upon nutritional therapeutic strategies for promoting body protein balance in stress or catabolic states. This might also be relevant to considerations of nutrient partitioning in animal food production. A recent review by the author and his colleagues (99) provides the detailed basis for much of what is covered in the following sections.

Energy dependency of protein and amino acid metabolism

To begin with, it is worth mentioning the major processes that require energy, in the form of high-energy phosphate bonds, involving ATP or GTP, and those which are intimately involved in the utilization of nitrogen and amino acids, in the formation of polypeptides and their further assembly within or outside the cell and, ultimately, in the breakdown and removal of proteins and the subsequent catabolism of amino acids. Hence, Table 1 lists many of the major processes and while these (and others not included here) are energy-dependent, it is, in most if not all cases, uncertain as to how much food energy is required to drive them in vivo (86). Therefore, it is not possible to assign, with any degree of confidence, the quantitative proportion of body-energy flux due to them, as we will again point out below. Nevertheless, it is these various processes that account, in a major way, for the molecular and cellular basis for the interactions between protein, nitrogen, energy metabolism, and their nutritional consequences.

Energy-nitrogen relationships, as evaluated from N-balance measurements

Body protein (nitrogen) balance is achieved, during normal growth or during maintenance in the case of the mature organism, within relatively narrow limits, indicating a regulation by varying the rate of N-excretion in relation to changes in nitrogen intake for a given metabolic and physiologic state. The responsiveness of nitrogen excretion to altered dietary conditions, especially nitrogen intake, was demonstrated many years ago. Indeed, the studies by Folin (32, 33) conducted at the turn of this century were, perhaps, the first significant examples of the impact of nutrition on a major metabolic pathway in the mammalian organism, in this case that of urea production. There have been numerous studies since then on the effects of protein and energy on body N balance and, in summary, the available data reveal that the effects of these nutritional factors are not entirely independent of one another and their interactions can be complex. As pointed out by Calloway and Spector (14), the level of caloric intake, whether above or below requirements, determines the changes in N-balance that can be achieved by an increase in N-intake. Furthermore, the level of N-intake determines the quantitative effect of energy intake on N-balance. Munro (59) captured this complexity quite clearly in his summary picture, which is reproduced in Fig. 1. The existence of separate

Table 1. Some energy-dependent processes associated with protein turnover and amino acid homeostasis[1]).

1. Protein turnover
   Formation of initiation complex
   Peptide bond synthesis
   Protein degradation
      Ubiquitin-dependent
      Ubiquitin-independent
      Autophagic degradation
      (sequestration, lysosomal proton pump)
2. RNA Turnover
      rRNA; tRNA
      pre-mRNA splicing (spliceosome) and mRNa
3. Amino acid transport
4. Regulation and integrity
      Reversible phosphorylation; enzymes, factors
      GTP-GDP exchange proteins (signal transduction)
      Second messengers (phosphatidyl inositol system)
      Ion pumps and channels
   ATP-dependent heat shock proteins (folding)
   Protein translocation
5. Nitrogen metabolism
      Glutamate/Glutamine cycle
      Glucose-alanine cycle
      Urea synthesis

[1]) From Young et al. (99)

protein and energy-dependent phases on N-balance or protein accretion has been demonstrated in lambs (e.g., (11)) and pigs (15), and a knowledge and understanding of these relationships and interactions is important for the design of diets that are efficient in terms of promoting protein retention, while achieving a desirable body and tissue composition.

In quantitative terms, for human subjects, excess energy intake at adequate levels of N-intake causes a retention of approximately 1–2 mg N per kcal in healthy subjects, but at limiting or low N-intakes this response is markedly attenuated (95, 99). At lower energy intakes, within the sub-maintenance range, the impact of changes in energy intake on N balance is greater; for normal young men it appears that at very low energy intakes the relationship between changes in energy intake and N balance is approximately 8 mg N $kcal^{-1}$, with this relationship breaking at a total energy intake of about 1400 kcal daily (14). This also holds for depleted, hospitalized patients (24, 25) in whom a value of 7.5 mg N/kcal has been found for energy intakes up to about 15 kcal/kg body weight (bw)/day. The metabolic explanation for this difference in the energy intake-N-balance relationship below and then above maintenance energy intakes is not yet certain. However, it is of possible interest that the "break-point" in the energy intake-N-retention relationship referred to above occurs at an energy level that is approximately equivalent to a glucose intake of approximately 3 mg $kg^{-1}min^{-1}$. This corresponds to about the level that maximally suppresses endogenous glucose production in the human adult (71). Therefore, presumably, the conversion of gluconeogenic precursors (including amino acids) for maintenance of glucose homeostasis is minimal at this glucose-intake level.

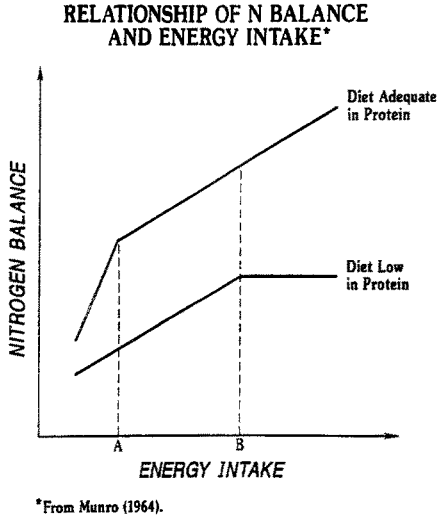

Fig. 1. Relationship of N-balance and energy intake with diets of differing protein levels. Between energy intake A (low) and B (higher) the two lines are parallel. Taken from Munro (59).

Table 2. Some estimates of protein synthesis in adults of various mammalian and avian species[1]).

Species	Body wt. (kg)	Daily protein synthesis ($g \cdot kg^{-1}$)	($g \cdot kg^{-0.75}$)
Rat	0.35	22	16.9
Rabbit	3.6	9.2	12.6
Wallaby (Parma)	4.2	7.5	10.8
Goat	38	6.6	16.4
Pig	32	8.1	18.9
Sheep	63	5.6	15.7
Man	71	4.6	13.4
Cow	575	3.0	14.8
Birds (chickens)	~1.4	27	30

[1]) Partial summary from Reeds and Harris (68) and Maramatsu (60), with addition of data from White et al. (90) for the wallaby.

Dynamic aspects of nitrogen/protein metabolism

Although a description of changes in N-balance with altered energy intake provides an initial insight into energy-nitrogen relations, it is now more useful to consider some dynamic aspects of body protein, amino acid, and nitrogen metabolism; we have probably now gained about as much physiological, mechanistically-based knowledge regarding protein-energy interactions as we can from nitrogen (N) balance data alone.

To begin with and, particularly, to justify further the present emphasis on studies in human subjects, it is useful to first compare whole-body protein turnover and its relation to energy expenditure. Thus, as shown in Table 2, rates of protein synthesis, when expressed per unit body weight, are high in the smaller mammalian species when compared to rates in larger mammals. However, when expressed to the three-quarter power of body wt ($kg^{0.75}$) there is a relative constancy among the various species listed here, although there are differences since the rate is considerably higher in avian as compared with mammalian species. In general, however, this summary indicates that protein synthesis is correlated with basal energy metabolism (BM), since the 0.75 power of body weight (in kg; "metabolic body size") is the weight function that approximately equalizes body energy expenditure across the different mammalian species (12). Therefore, based largely on Waterlow (85), it can be seen in Table 3 that the change in the intensity of body protein turnover parallels that for differences in their metabolic rates (kJ); expressed as kJ metabolic rate per g protein turnover the values shown is Table 3 range from 11–23, with a mean of about 15. Of possible interest is that the lower rate of protein turnover in marsupials is also associated with a lower metabolic rate (90), such that the basal energy expenditure per unit of protein synthesis approximates that for eutherian mammals. Similarly, the higher rate of protein turnover in chickens is paralleled by a higher basal or resting metabolic rate; it can be estimated (Table 3) that approximately 16 kJ resting energy expenditure are associated with each g of protein synthesis

Table 3. Whole body protein turnover in relation on resting metabolic rate (MR) in adults of mammalian and avian species[1]).

Species	Wt. (kg)	Protein turnover ($g \cdot kg^{-1}\ day^{-1}$) –A–	MR ($kJ \cdot kg^{-1}\ day^{-1}$) –B–	Ratio B/A
Mouse	0.04	43.5	760	11
Rat	0.35	22.0	364	17
Rabbit	3.6	9.2	192	20
Wallaby (Parma)	4.2	7.5	163	21
Sheep	63	5.6	96	17
Man	70	4.6	107	23
Cow	575	3.0	60	20
Birds (chickens)	1.4	27	439	16

[1]) From Waterlow (85), with addition of data for birds from Muramatsu (60) and White et al. (90) for the wallaby.

in this species. This is again similar to that for mammals. Therefore, from these various data it appears that, on average, about 15–20 kJ of basal energy expenditure are "connected" with each g protein synthesis. Assuming, therefore, that about 3 kJ are expended (minimally, in the formation of peptide bonds) per g protein synthesis (86), this implies that approximately 20 % of basal metabolism is due to the process of polypeptide chain elongation. However, this represents an underestimate of the total energy cost of protein synthesis because, as already stated, it is not yet possible to assign quantitative estimates of the energy needs of the various processes responsible for bringing about the formation of proteins and their eventual positioning within their functional intracellular, membrane or extracellular regions. Furthermore, it is likely that the significant energy cost of establishing the Na^+ electrochemical gradient across the cell membrane (e.g., (44)), which provides the driving force for the A system of amino acid transport, should also be considered as an energy cost of protein turnover.

The point to consider here, however, given the general similarity in the association between protein synthesis and basal energy metabolism among these various species, is whether the metabolic responsiveness to altered protein and energy intakes and ratios is also broadly comparable between man and the livestock species of interest here. There are, unfortunately, limited data to permit a definitive comparison among different species, but it seems that the same general principles might apply. For example, Muramatsu (60) has reviewed the relationships between dietary protein and metabolizable energy intake, growth and protein retention, and whole-body protein synthesis in young chicks and has compared these with the more limited data available in non-human mammalian species. He concludes that the responses of whole-body protein turnover for avian and mammalian species are not evidently different, suggesting comparable underlying mechanisms. The precise quantitative aspects of the relationships between protein and energy intakes and metabolism would be expected to vary according to the particular species, develop-

mental state and age, genotype and environmental conditions. However, for the present purpose, I will accept Muramatsu's (60) conclusion as further justification for my choice to review mainly studies of protein and energy interactions in humans.

Clearly, changes in energy intake effect alterations in the utilization of dietary amino acids. Thus, it is important to understand in greater detail how the source and level of energy intake affects the metabolic and organ fate of specific dietary amino acids, and we will examine this question in more detail later. Thus, to begin, it would be worthwhile knowing whether the fate of absorbed amino acids, such as leucine, during their passage through the splanchnic region differs according to the level and source of carbohydrate and/or lipid in meals. Indeed, a detailed exploration of the metabolism of individual amino acids following their absorption represents an area of fruitful investigation in both non-human models, as well as in man. This might be undertaken, in part, as we have done by exploiting multiple stable isotope tracer protocols, with simultaneous intravenous and intragastric administration, to quantify so-called "first pass" effects (e.g., (45)).

For example, in a recent study in young adults, we (50) applied this intravenous-intragastric-tracer approach to explore the metabolic fate of dietary leucine, when the tracer was incorporated into a balanced amino acid mixture and which was consumed in the presence or absence of carbohydrate (as glucose). We found that inclusion of the energy substrate with the experimental meal reduced the "first-pass" disappearance of leucine and this response was associated with a lower rate of whole-body leucine oxidation (Table 4). Hence, the dietary addition of glucose appeared to promote a greater passage of dietary leucine to peripheral tissues where it was, presumably, used to an increased extent for protein anabolic purposes. This response, however, was not observed for labeled phenylalanine disappearance which reveals, perhaps, that the specific effect of glucose addition will depend upon the test amino acid and its particular organ-metabolic structure. These results indicate the need to probe further, with the aid of tracer techniques, the metabolic basis for changes in nitrogen balance due to alterations in the dietary energy supply.

Table 4. Effect of an amino acid meal, with and without carbohydrate (CHO), on leucine kinetics studied with 1-^{13}C-leucine given i.g. and $^{2}H_3$-leucine given i.v.[1]).

Measurement	Amino acids alone	Amino acids + CHO
Leucine flux (i.g. tracer)	188[2]	158*
Leucine oxidation	43[2]	36*
Leucine splanchnic removal (%)	29	20*
Leucine release protein	130	100*
Net leucine balance	+14	+20*

[1]) From Krempf et al. (50)
[2]) Values are $\mu mol \cdot kg^{-1} \cdot h^{-1}$.
* Different from amino acids alone; $p < 0.05$.

Table 5. Obligatory oxidative losses (OOL) of amino acids and intakes required to balance these in adult subjects[1]).

Amino acid	OOL	Intakes for Balance (A)	FAO/WHO/UNU (1985) (B)	Ratio A/B
		$mg \cdot kg^{-1}\ day^{-1}$		
Isoleucine	16	23	10	2.3
Leucine	27	39	14	2.8
Lysine	30	42	12	3.5
SAA	13	16	13	1.2
AAA	27	39	14	2.8
Threonine	15	21	7	3.0
Tryptophan	4	6	3.5	1.7
Valine	17	24	10	2.4

[1]) Based on Young et al. (96)

Obligatory N and amino acid oxidative losses; relation to requirements

While we are considering the dynamics of protein and amino acid metabolism in relation to basal expenditure it is of possible interest to mention that differences in rates of endogenous N-loss among the various mammalian species are also related to their basal energy expenditure, a relationship described by Brody (13) more than four decades ago. At this juncture, therefore, I wish to consider briefly how these losses might be used as a basis for predicting the requirements for specific indispensable amino acids. This emerges from a suggestion made by Millward and Rivers (56) that minimum rates of oxidative losses of the indispensable amino acids might be estimated from obligatory nitrogen losses. We were attracted by this idea and extended it as a basis for predicting the minimum physiological intakes (requirement) necessary to maintain body amino acid balance in the healthy, well-nourished subject. The approach that we have taken has been described in detail (96) and it is analogous to the factorial method used by the FAO/WHO (26) Expert Committee on Energy and Protein Requirements for estimating the total N (protein) requirement of individuals at various ages. While the factorial approach was abandoned by a more recent FAO/WHO/UNU (27) Expert Consultation, for purposes of arriving at dietary protein recommendations, we considered it to be a useful procedure for an initial reassessment of the indispensable amino acid requirements in the adult humans.

An additional impetus to explore this procedure comes from an appreciation for the importance of the status of the oxidative catabolism of amino acids as a determinant of requirements. An interesting and relevant example in this context emerges from the recent amino acid kinetic studies by Thompson et al. (80, 81) in patients with maple syrup urine disease (MSUD), who have impaired branched chain α-ketoacid dehydrogenase (BCKADH) activity due to mutations in different regions of the E_1 and E_2 components of the BCKADH, mitochondrial multienzyme complex (e.g., (63)). These (80, 81) investigators showed that, despite significant eleva-

tions of plasma leucine in MSUD subjects, mean rates of whole body proteins synthesis and catabolism were similar to control values, as was also, and importantly, their growth rates. However, leucine oxidation rates in the MSUD subjects were about 5 to 10 times lower than the rates expected in healthy subjects who receive adequate, but not excessive, intakes of leucine. This is a clear demonstration that the status of tissue oxidative catabolism is a primary determinant of the requirement value for this indispensable amino acid.

Therefore, we (96) have estimated obligatory oxidative losses (OOL) of amino acids and minimum intakes to balance these in human subjects, and these are given in Table 5. As can be seen, these predicted, required intakes are two to three times those accepted by FAO/WHO/UNU (27) as being at the upper range of the minimum requirement for this age group.

Millward et al. (57), in criticizing our approach, stated ". . . it can be in no way justifiable to take values for the OOL as the basis for the new requirement pattern as suggested by Young et al. (96)". However, we believe that there is some justification for our approach and, further, that the evidence used as a basis for the criticism developed by Millward et al. (57) has its own significant limitations. Hence, it is worth critiquing their approach and conclusion, as well as seeking further support for the foregoing use of OOL and the new requirement estimates obtained from the OOL data (Column 2, Table 5).

First, we turn to a brief discussion of the amino acid requirements in preschool-age children that were established using multiple criteria, including N-balance and plasma amino acid levels, by workers at INCAP in Guatemala (65). Thus, it would be of interest to learn whether it is possible to predict, with reasonable accuracy, these requirements from OOL, taking also into account the amount of amino acids deposited as net body protein gain. I have made these calculations and they are summarized in Table 6. For the three branched chain amino acids, for example, the requirements for the 2–5 years age group are predicted to be approxi-

Table 6. An estimate of the amino acid requirements of 2-year-old children as derived from obligatory N losses and an assumed efficiency of amino acid retention.

Amino acid	Body protein[1] (g/16gN)	Obligatory loss (OL)[2]	To balance OL[3]	Deposited[4]	Total required
			$mgkg^{-1}day^{-1}$		
Leucine	6.9	27.6	39.4	13.4	52.8
Isoleucine	4.1	16.4	23.4	8.0	31.4
Valine	5.0	20.0	28.6	9.7	38.3
Lysine	7.8	31.2	44.6	15.1	59.7
Threonine	4.2	16.8	24.0	8.1	32.1
Aromatic	7.6	30.4	43.4	14.7	58.1
Sulfur	3.4	13.6	19.4	6.7	26.1

[1]) From Reeds (67). [2]) Assuming 64 mgN/kg/day for total obligatory N losses (FAO/WHO/UNU, 27). [3]) Assuming 70 % retention of amino acids when consumed at "requirement" levels. [4]) Net deposition based on a value of 31 $mgN/kg^{-1}day^{-1}$ for the 2-year-old child (FAO/WHO/UNU, 27).

Table 7. Comparison of amino acid requirements in 2-year-old children: estimations from obligatory oxidative losses (OOL) with those of FAO/WHO/UNU/1985.

Amino acid	Requirements based on:		
	Predictions from OOL (A)	1985 FAO/WHO/UNU (B)	Ratio A/B
Leucine	53[1])	73[1])	0.7
Isoleucine	31	31	1.0
Valine	38	38	1.0
Lysine	60	64	0.94
Threonine	32	37	0.86
Aromatic	58	69	0.84
Sulfur	26	27	0.96

[1]) Values are mg $kg^{-1}day^{-1}$ (see Table 6)

mately 53, 31, and 38 mg $kg^{-1}day^{-1}$ for leucine, isoleucine, and valine, respectively. When these, as well as the predictions for the remaining indispensable amino acids (Table 6), are compared with the FAO/WHO/UNU (27) figures, it is evident that the predicted requirement values are reasonably close to those proposed by the international expert consultation (Table 7). Indeed, the agreement is rather remarkable, considering that the directly determined values are based only on a single series of experiments carried out by Pineda and coworkers (65). It appears, therefore, that the requirements for preschool children can be predicted from OOL.

Second, Millward et al. (57) have largely based their arguments on the amino-acid requirement estimates for maintenance and growth, as

Table 8. Pattern (relative to methionine and cystine) of amino acids required for maintenance or growth in young pigs, compared with pattern of obligatory oxidative losses (OOL).

Amino acid	Growth[1]) (G)	Maintenance[1]) (M)	OOL[2])	OOL/G	OOL/M
Threonine	1.3	1.1	1.1	0.8	1.0
Valine	1.5	0.4	1.3	0.9	3.3
SAA	(1.00)	(1.00)	(1.00)	–	–
Methionine	0.5	0.2	–	–	–
Isoleucine	1.2	0.3	1.2	1.0	4.0
Leucine	2.2	0.5	2.0	0.9	4.0
AAA	2.4	0.8	1.1	2.2	1.4
Phenylalanine	1.1	0.4	–	–	–
Lysine	1.9	0.7	2.2	0.9	3.1
Tryptophan	0.3	0.2	0.3	1.0	1.5

[1]) Calculated from Table 3 in Fuller et al. (40). [2]) Calculated from Table 2 in Millward and Rivers (56)

reported by Fuller et al. (40) for the growing 40–50 kg bw pig. Hence, Millward et al. (57) have pointed out that the pattern of the obligatory oxidative losses is more like the dietary pattern of amino acids required for growth than for the amino acid pattern that is apparently required for maintenance in this animal model (see Table 8). From such a comparison, these investigators (57) accept that: "the pattern of the OOL *must (present author's emphasis)* be quite different from the minimum requirement pattern." However, we consider this conclusion to be based on a flawed, quantitative extrapolation to the human nutritional context from observations made with a pig model. Our reasoning for this is as follows: First, the proportionate contributions to the total amino acid (and nitrogen) requirement due to maintenance and that for growth are profoundly different for the growing pig as compared to both the young child and human adult. As shown in Fig. 2, taken from the data of Carr et al. (16), the growth component accounts for about 90 % of the total requirement in the 40-kg pig, and it continues to remain high as the animal progresses towards a mature weight (> 140 kg). In the human subject, in contrast (Fig. 3), the growth component of the total protein requirement is considerably lower than for the growing pig, being about 50 % at 6 months of age, and then declining to about 20 % in the 2-year-old child, and 15 % or less in the 4–5 year old. Thus, on this basis alone, it appears that the quantitative importance of the metabolic processes responsible for the amino acid requirement in the growing pig differs profoundly from that for the preschool child. It must be questioned, therefore, whether amino acid requirement estimates in the growing pig provide a legitimate basis from which to criticize predictions of human amino acid requirements as derived from OOL. Our view is that the valuable data of Fuller et al. (40) cannot be used

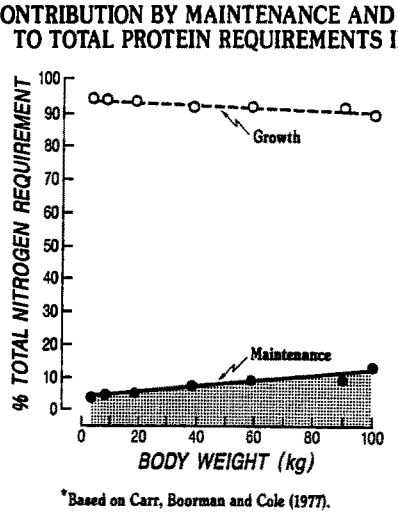

Fig. 2. Contribution made by maintenance and growth to the total protein (nitrogen) requirement in pigs at various body weights. Calculated and drawn from tabular data of Carr et al. (16).

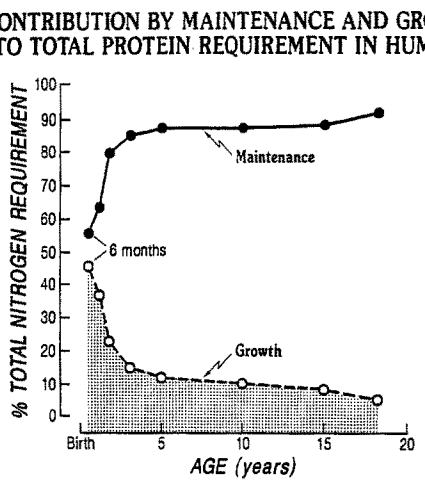

Fig. 3. Contribution made by maintenance and growth to the total protein (nitrogen) requirement of human infants, young and older children. Calculated and drawn from data presented by FAO/WHO/UNU (27).

to resolve problems concerning *quantitative* aspects of human amino acid requirements.

Third, we have compared the pattern of the amino acid requirement for the preschool child, as proposed by FAO/WHO/UNU (27), with the composition of mixed proteins in the body. As shown in Table 9, when the amino acids are expressed in relation to the requirement for, or content of, the sulfur amino acids (which, according to Millward and Rivers (56), may be the rate-limiting amino acids for driving the obligatory nitrogen losses), it is clear that there is a close similarity between these two patterns,

Table 9. Comparison of pattern of amino acid in body protein with requirement pattern of amino acids for preschool child.

	mg/g Crude protein			Relative to Methionine		
Amino acid	Body protein[1]) (A)	Pre-school[2]) (B)	Ratio B/A	Body protein (A)	Pre-school (B)	Ratio B/A
Isoleucine	41	28	0.7	1.2	1.1	0.92
Leucine	69	66	1.0	2.0	2.6	1.3
Lysine	78	58	0.7	2.3	2.3	1.0
Met and cys	34	25	0.7	1.0	1.0	(1.0)
Phe and tyr	76	63	0.8	2.5	2.5	1.1
Threonine	42	34	0.8	1.4	1.4	1.2
Tryptophan	12[3])	11	0.9	0.4	0.4	1.0
Valine	50	35	0.7	1.4	1.4	0.9

[1]) From Table 1 in Reeds (67). [2]) From Table 38 in FAO/WHO/UHU (27). [3]) For beef protein (FAO/WHO/UNU, 27).

despite the quantitative importance that maintenance makes to the amino needs of the 2-year-old child, as discussed above. It follows, then, that the amino acid pattern for meeting the need in the 2-year-old child, which is dominantly a need for maintenance, is similar to the pattern of amino acids in body protein or that of the OOL.

Fourth, it is apparent that the efficiencies of dietary nitrogen utilization, and presumably of dietary amino acid retention, at maintenance intakes in the 40-kg pig (Millward's model for man) and the 2–5-year-old child are substantially different. According to Fuller et al. (40), the young pig uses dietary nitrogen with an approximate 100 % efficiency for maintenance. In the young child, it is substantially less than this, being about 70 % according to the FAO/WHO/UNU (27). Again, quantitative features of body nitrogen metabolism in relation to maintenance in the growing pig and young child are very different.

Fifth, and finally, I wonder about the physiologic, as well as nutritional, significance of "maintenance" amino acid needs in the growing organism, since at these intake levels the organism would effectively be receiving a deficient intake in relation to its genetically-determined metabolic potential. Furthermore, it will be of interest to learn whether the maintenance amino acid requirement pattern proposed by Fuller et al. (40), based on studies in the growing pig, is capable of efficiently maintaining protein homeostasis in the mature or minimally growing pig. This issue is also raised because estimates of the maintenance requirements for threonine, sulfur amino acids isoleucine and lysine in 145 kg gilts (e.g., (4, 5)) do not agree with those proposed by Fuller et al. (40). Indeed, the differences between these various estimates may be as much as twofold, thus weakening the argument and conclusions drawn by Millward et al. (57).

To summarize thus far: i) the amino-acid requirements predicted from OOL agree quite well with those determined from metabolic studies in 2-year-old children; ii) the pattern of the amino-acid requirement derived from metabolic studies in this young age group is, of course, similar to the composition of the mixed proteins in the body, despite the large maintenance and small growth components, to the total requirement for nitrogen and amino acids; iii) the arguments made by Millward et al. (57) against use of OOL for, at least, an approximation of human amino acid requirements are questionable due to major differences in the quantitative characteristics of nitrogen and amino acid nutrition in the growing pig as compared with humans beyond very early infancy; iv) the prediction of adult amino acid requirements from OOL appears, therefore, to have merit.

It would be desirable to seek further support for the conclusion, drawn from the foregoing predictions (Table 5) that the adult human amino acid requirements are up to two to three times higher (except for the S-amino acids) than those based on earlier N-balance studies (26, 27). Support has, indeed, been obtained from a series of studies conducted in our laboratories in which we explored use of stable nuclide labeled amino acids, with measurements of amino acid oxidation in healthy adult subjects given defined dietary treatments (e.g., (96, 97, 98)). Our experiments were conducted on the premise that a direct measure of the oxidation of a specific indispensable amino acid (coupled with a determination of the

minimal intake to balance this oxidation) in well-nourished, normal individuals would yield a more satisfactory estimate of the minimum physiological requirement for the amino acid than is possible from N-balance measurements.

Table 10 gives our estimates of amino acid requirements from ^{13}C-tracer experiments and compares these values with those predicted from OOL (Table 5). In view of the difficulties encountered in the design, protocol, and interpretation of amino acid kinetic studies, and the reasonable, but not necessarily valid assumptions concerning amino acid efficiency and retention applied in making the predictions, there is a remarkably good agreement between the estimated requirement levels for the five amino acids listed here.

I have given, above, some emphasis to obligatory losses and amino acid requirements for a number of major reasons, in reference to the present topic of protein-energy interactions. The first was that differences in nitrogen metabolism, viewed either in the context of protein turnover or as obligatory urinary nitrogen losses, among various mammalian species parallel differences in their energy metabolism. Because the obligatory N-losses account for a major proportion of the requirement for dietary nitrogen, at least for maintenance, then the requirement for protein would also bear a close and possibly functional relation with resting energy metabolism. Furthermore, the requirements for the individual, indispensable amino acids account for a quantitatively important proportion of the need for total nitrogen, and so it was of interest to attempt to relate obligatory N-losses to amino acid requirement estimates. A second reason for this focus on human amino acid requirements and OOL was because I believe it is useful to apply knowledge about comparative aspects of physiology and nutrition to extend our understanding of metabolic and nutritional issues and processes, within a particular genetic context. Simultaneously, however, it is necessary to remain alert to the limitations of this approach, especially when trying to establish, by extrapolation from one species to another, quantitative characteristics of protein/amino acid and energy metabolism/nutrition for a particular situation, such as for the human.

Table 10. Comparison of ^{13}C-amino acid derived estimates of requirements in adults.

Amino acid	^{13}C-tracer[1])	Predicted[1]) (OOL)	FAO/WHO/ UNU 1985[2])
		$mg \cdot kg^{-1}day^{-1}$	
Leucine	40	39	14
Valine	20	24	10
Lysine	30	42	12
Threonine	15	21	7
Met (-Cys)	13	16	13

[1]) Approximate *mean* requirement. See Young et al. (96) for background.
[2]) This is the *upper range* of the requirement for adults.

A third reason for this brief critique of amino acid requirements, a topic of long-term research interest to this author, was because of the sensitivity of N-balance to altered energy intake. Indeed, we have concluded previously (96) that many of the earlier estimates of the amino acid requirements in human adults have been obtained from studies in which the interactions between protein and energy on N-balance have confounded the interpretation of the N-balance data. These earlier studies, therefore, must be viewed cautiously, especially when attempting to arrive at a quantitative definition of human amino acid requirements.

So far, we have discussed some aspects of protein and amino acid metabolism in relation to total energy intake and metabolism without specific reference to energy source. Hence, at this point it would be worthwhile to turn to a consideration of the effects of carbohydrate and lipid-based energy substrates on some aspects of protein metabolism/nutrition.

Energy source and the nitrogen economy

Glucose and lipid effects

It is of both metabolic and practical, nutritional interest to determine whether the different energy-yielding substrates exert similar or specific effects on protein utilization. Hence, the in vivo metabolic basis for their effects deserves our attention and, in particular, because there is not an obligatory requirement for dietary carbohydrate. Some tissues normally show a preference for glucose as a major fuel (brain, erythrocytes, renal medulla), but this can be obtained via hepatic gluconeogenesis from amino acids and from the glycerol released by hydrolysis of triacylglycerol. It would be expected under conditions of a very low, or devoid, carbohydrate intake that gluconeogenesis would be high. Thus, a consequence of this would be a relative increase in nitrogen excretion and a decline in the overall efficiency of dietary N retention.

Studies conducted earlier in this century by Cathcart (18) revealed that a relatively small carbohydrate load (about 400 kcal/day) markedly reduced the loss of N in fasted subjects. He also observed that an isocaloric level of fat did not have this same N-sparing effect (e.g., Fig. 4). These early findings have been extended through more elegant studies of leucine oxidation, both before and after obese volunteers consumed low-energy diets, based on carbohydrate or fat, or a combination of the two. Thus, Vasquez et al. (84) found that, during caloric restriction, dietary carbohydrate reduced the catabolism of leucine. Lipids, on the other hand, increased leucine oxidation. Assuming that the latter reflects the status of overall amino acid (or nitrogen) catabolism, these early and more recent findings are entirely consistent and suggest that we might continue with a brief discussion of carbohydrate versus lipid effects based on N balance data.

As stated by Munro (59), carbohydrate has specific actions on protein metabolism and N-balance not equally shared with fat; administration of carbohydrate lowers body N-output in the fasting subject, whereas fat does not (and the isocaloric substitution of fat for carbohydrate results in a transient increased N-output). Also, separation of meals containing dietary

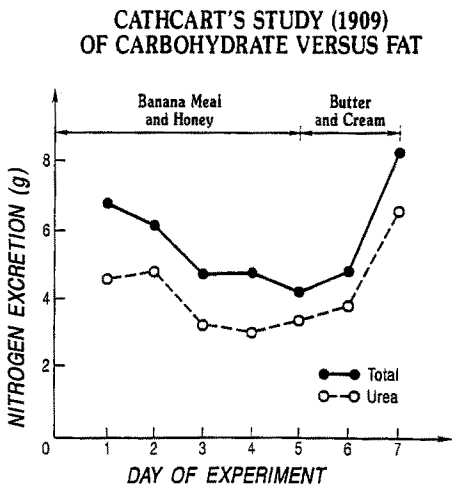

Fig. 4. Urinary nitrogen excretion for an adult human subject who consumed a low-nitrogen, "high"-carbohydrate diet (consisting of banana meal and honey) for 5 days followed by a high fat diet (consisting of 65 g butter and 340 g 55 % cream) for 2 days. Drawn from tabular data of Cathcart (18).

carbohydrate from those containing protein causes a transient rise in N-output, whereas this does not occur when fat is separated from protein intake. From these findings, Munro (59) concluded that carbohydrate exerts a specific action on the utilization of amino acids during their absorption from protein-containing meals, in addition to an effect shared by fat and carbohydrate on the metabolism of amino acids during the postabsorptive period.

Because the practical, protein-nutritional significance of many of the earlier N-balance studies was difficult to judge, especially for those carried out at supramaintenance intakes of protein, we (69) also studied the quantitative effect on protein utilization at barely adequate protein intakes of an isoenergetic exchange of dietary fat for carbohydrate in healthy young men. We found that nitrogen (N) balance and dietary protein utilization were significantly higher with the carbohydrate diet. Also, relevant was the observation that the protein-sparing effect of the carbohydrate was greatest in those subjects who were on marginal energy and protein intakes and who were losing weight. How this response of N-etabolism relates to the different capacity for carbohydrate versus lipid oxidation at adequate-to-surplus levels of energy intake (2) remains an interesting question. Also, whether or not these "longer-term" effects of a change in dietary carbohydrate: fat ratio on N-metabolism are mediated through the action of insulin (as Munro (59) postulated to explain results for acute experiments) remains to be determined. However, some observations would support the contention that the favorable effect of dietary carbohydrate on body N-balance is mediated, in part, by the effects of insulin. Thus, Fuller et al. (39) used a continuous infusion of physiological amounts of exogenous insulin together with glucose in well-nourished pigs. The glucose was infused with insulin at a rate sufficient to maintain

plasma glucose concentrations within the normal physiological range, and to avoid the normal hypoglycemic and counterregulatory response in body metabolism. The response to this treatment over a period of 3 to 7 days was a two- to seven-fold increase in plasma insulin, a 50 % decrease in plasma glucose, a 40 % decrease in plasma urea concentration, and a 30 % fall in urinary excretion of urea N. After terminating the insulin infusion, plasma urea levels rapidly returned to those of the control period. These authors (39) concluded that a major component of the protein-sparing effect, achieved by a surfeit feeding of carbohydrate, is mediated by insulin.

We will return to this point below, but the foregoing suggests that at high sub-maintenance or adequate energy intakes there is a slightly greater sparing effect on overall N retention due to carbohydrate, compared with fat, when total N intakes are marginally adequate. However, at more generous N intakes and with initially adequate energy intakes, the protein-sparing effects of glucose and lipid would appear to be essentially comparable and of little or no significance for human protein nutrition, per se. I would be interested in knowing whether these same principles apply to the growing pig and domestic chick.

Possible physiologic mechanism for N-sparing

Even if glucose and fat have an equivalent impact on whole body N-retention in humans, when intakes of glucose are non-limiting (or above that necessary to maximally suppress endogenous glucose production), it remains desirable to learn more about the mechanistic basis for their N-sparing effects.

An indication that glucose and lipid exert their effects on N-balance via different mechanisms comes, for example, from Baker et al. (6), who found differences in blood plasma amino acid and hormonal profiles between lipid and glucose-based systems, and from Shaw and Holdaway (73), who found that lipid did not affect glucose oxidation and production, whereas glucose did. Also, Ferranini et al. (30) found that lipid infusion in healthy volunteers had a hypoaminoacidemic effect of its own and distinct from that due to glucose. Again, this indicates differences between glucose and lipids in terms of their specific metabolic effects, despite a possible similarity in their impact on total body N-balance. The fate of amino acids and detailed aspects of their metabolism can only be partly understood by observing changes, or differences, in plasma amino acid levels. Thus, we turn again to some tracer studies of whole body and regional amino acid kinetics in order to gain a better appreciation for the comparative metabolic effects of carbohydrate and lipid fuels on the nitrogen economy of the host.

Emphasis on glucose, insulin, and other hormones

Because changes in energy and nitrogen metabolism, and in their relations, are undoubtedly dependent upon a complex of inter-relate mechanisms involving, in part, the hormonal regulation of substrate mobilization, utilization, and interconversion, it is necessary to examine energy-

nitrogen relations in reference to a number of key hormones. Among these, insulin is taken to be of particular importance.

In general, the net effects of insulin on whole-body protein regulation appear to be a distinct decrease in proteolysis and a stimulation (perhaps slight) of protein synthesis, although there have been differences, both qualitative and quantitative, in the reported findings.

Hence, Table 11 presents a selected survey of studies concerned with whole-body amino acid (mainly leucine) kinetics in adults and the effects of insulin or glucose and/or amino acid administration i.v. It is reasonable to conclude from the listing of results given in this table that whole-body leucine (and tyrosine) kinetics are sensitive to insulin and glucose administration and that insulin generally exerts an inhibitory effect on protein breakdown which ins often associated with a reduced rate of amino acid oxidation. This fall in whole-body protein breakdown occurs in an insulin dose-dependent manner (36, 76). Because skeletal muscle is a tissue that is sensitive to insulin action, this reduction in whole-body protein breakdown might be attributed, at least, to a decrease in the output of amino acids from the skeletal muscles.

A summary of findings from a number of investigations concerned with the effects of insulin on in vivo aspects of protein and amino acid metabolism in the skeletal musculature of adult humans is presented in Table 12. It is evident that the consistency of the observations regarding insulin's suppression of whole-body proteolysis (Table 11) do not extend to the in vivo studies on skeletal muscle (Table 12); some have found a decrease in muscle protein breakdown, whereas others have not, despite an improved overall amino nitrogen balance across the organ when insulin and glucose are given. Whether these variable findings are due to the fact that different tracer models have been used, whether the insulin is given locally or systemically, or whether forearm responds differently the hindlimb, for example, is not at all clear. Also, there is still little in vivo evidence that insulin stimulates, to any major extent, muscle protein synthesis. A recent study by Bennet et al. (10) indicates that insulin promotes synthesis when the availability of amino acids is not limiting.

Thus, these various investigations provide an incomplete and somewhat uncertain picture, but it should be also recognized that the effect of insulin on the overall body protein economy depends upon whether an exogenous amino acid supply accompanies insulin administration. When amino acids are supplied, whole-body (38, 78) and muscle protein (10) synthesis, which require available amino acids, may be stimulated. Nevertheless, the effect of insulin in stimulating synthesis appears to be small. Also, insulin's suppression of proteolysis appears to be augmented by amino acids (31).

In general, although somewhat tentative in terms of specific mechanisms, the various actions of insulin and glucose on leucine (amino acid; protein) kinetics are consistent with the fact that a maximum nitrogen retention is achieved when both protein and carbohydrate intakes are consumed together at generous protein intakes. However, the relations between insulin, carbohydrate, and amino acids are probably more complex than this discussion implies. Basically, the quantitative, and even qualitative role played by insulin in relation to the underlying components

Table 11. A selected survey of effects of insulin, glucose and amino acids on in vivo amino acid kinetic in human adults: whole body.

Authors	Tracer approach	Conditions	Outcome
Robert et al. (71)	Continuous ^{13}C-leu	Glucose alone; insulin; euglycemic	PS ↓; PB ↓ Leu ox–
Fukagawa et al. (36)	Continuous ^{13}C-leu	Euglycemic insulin clamp	PS ↓; PB ↓ Leu ox–
Wolfe et al. (92)	Continuous ^{13}C-ala and ^{15}N-urea	Glucose infusion with constant insulin and glucagon	Ala flux ↑ Urea production↓
Tessari et al. (76)	Continuous ^{3}H-leu; ^{14}C-kic	Post-absorptive: Variable insulin with euglycemia	PS ↓; PB ↓; Leu ox ↓
Fukagawa et al. (37)	Continuous ^{13}C-leu and ^{15}N-ala	Post-absorptive; euglycemic insulin clamp	PB↓, Leu ox–
Tessari et al. (79)	Continuous ^{3}H-leu; and ^{14}C-kic	Type 1 diabetics insulin without glucose	↓ PB
Shangraw et al. (72)	Continuous ^{13}C-leu	Post-absorptive; euglycemic insulin clamp	↓ PB; leu ox ↓
Castellino et al. (17)	Continuous ^{14}C-leu	Insulin with low, basal and elevated plasma amino acids	↓ PB ↓ Leu ox
Arfvidsson et al. (3)	Continuous U-^{14}C-tyr	Post-absorptive: insulin with euglycemic	Tyr flux ↓
Tessari et al. (78)	Continuous ^{3}H-leu; ^{14}C-kic	1. Hyperinsulinemia with euglycemia 2. Hyperaminoacidemia 3. Hyperaminoacidemia with euglycemic hyperinsulinemia	↓ PB ↓ PS; ↓ PB
Fukagawa et al. (38)	Continuous ^{13}C-leu	Amino acids alone AA with euglycemic hyperinsulinemia	↓ PS ↓ PS; ↓ PB
Flakoll et al. (31)	Continuous ^{14}C-leu	Insulin with hypoaminoacidemia Amino acids plus insulin with euglycema and euaminoacidemia	↓ PS; ↓ PB ↓ ↓ Leu ox ↓ PB; PS-
Frexes-Steed et al. (34)	Continuous ^{14}C-leu	Post-absorptive and 4-day fast. Variable leucine with euglycemia and euleucinemia	↓ PB

PS = protein synthesis; PB = protein breakdown; Leu ox = leucine oxidation; ↓; ↑; – decrease, increase, no change, respectively.

Table 12. A selected survey of effects of insulin, glucose and amino acids on in vivo amino acid kinetics in humans: muscle.

Authors	Model	Conditions	Outcome
Pozefsky et al. (66)	Forearm: A–V balance	Local hyperinsulinemia	↑ AAN balance
Elia et al. (23)	Forearm: A–V balance	Glucose infusion	↓ Ala output; ↑ AAN balance
Gelfand and Barrett (43)	Forearm: ^{3}H-phe; 1–^{14}C-leu exchange	Local hyperinsulinemia	↓ MPB
Bennet et al. (9)	Anterior tibialis; ^{13}C-leu; biopsy	Fasting and amino acids	↑ MPS with amino acids
Bennet et al. (10)	Leg: A–V difference ^{15}N-Phe; 1-^{13}C-leu;	AA alone AA + insulin with euglycemia	Insulin augmented leu uptake; MPB ?
Fryburg et al. (35)	Forearm: ^{3}H-phe; ^{14}C-leu	12 + 60 h fast + local insulin	Insulin ↓ MPB
Tessari et al. (79)	Forearm: ^{3}H-leu; ^{14}C-kic	Type 1 diabetic; Peripheral insulin and amino acids	No effect on muscle
Arfvidsson et al. (3)	^{14}C-tyr: leg exchange	Physiologic hyperinsulinemia	MPS – MPB –

AAN = amino acid nitrogen; MPB, MPS = muscle protein breakdown and synthesis, respectively
↑, ↓, – = increase, decrease or no change, respectively

of the N-balance responses discussed above cannot yet be stated with any degree of clarity.

In relation to the overall anabolic action of insulin on body protein balance, it is also pertinent to question the possible involvement of the counter regulatory ("anti-insulin") hormones, glucagon, catecholamines, and the glucocorticoids in energy-nitrogen relations. For example, ingestion of carbohydrate suppresses and protein intake stimulates glucagon secretion. It is also clear that the circulating glucagon concentration depends on the ratio of protein to carbohydrate in meals (e.g., (51, 89)).

As summarized in Table 13, when glucagon is infused during a state of insulin deficiency, there is an increased rate of whole-body leucine oxida-

Table 13. Effects of various hormones on in vivo amino acid kinetics in adult humans.

Author	Model and approach	Outcome
Glucagon		
Nair et al. (62)	^{13}C-leu infusion: glucagon with insulin deficiency	↑ PB; ↑ leu ox
Couet et al. (20)	^{13}C-leu infusion: glucagon excess with maintenance of insulin	PB–
Epinephrine		
Miles et al. (55)	^{15}N-leu; ^{2}H-ala; ^{2}H-leu and epinephrine infusion	↓ leu ox; ↓ PAA
Lamont et al. (52)	β-adrenergic blockade (propanolol 80 mg bid) 1-^{13}C-leu infusion	↑ leu ox; PB- urea N/creatinine ↑
Kraenzlin et al. (49)	^{13}C-leu infusion epinephrine infusion with constant insulin & glucagon	↓ PB; ↓ leu ox; But net forearm leu release ↑
Matthews et al. (53)	Infusions of ^{13}C-leu; $^{2}H_5$-phe; ^{15}N-glu(NH_2); epinephrine infusion for 8.5 h	PB–; little change in ↑ glu(NH_2) flux; leu ox–; ↓ PAA
Glucocorticoids		
Gelfand et al. (42)	^{13}C-leu; 72h infusion cortisol (6 mg/m^2 per h)	leu flux– leu ox–
Simmons et al. (75)	$^{2}H_3$-leu; 8 h hydrocortisone infusion	↑ PB
Darmaun et al. (21)	^{13}C-leu; $^{2}H_5$-phe; ^{15}N-glut (NH_2); ^{13}C-ala; hydrocortisone infusion for 64 h	PB ↑, Ala flux ↑ Glut(NH_2) flux ↑
Beaufrere et al. (8)	^{13}C-leu; ^{15}N-glu(NH_2); ^{13}C-ala; 20 mg 3× daily prednisone	↑ leu ox; ↑ PB ↑ glu(NH_2) flux & syn ↑ ala flux & syn

PB = whole body protein breakdown; PAA = plasma amino acid concentration; leu ox = leucine oxidation; ↑, ↓, – = increase, decrease or no change

tion and proteolysis (61). Thus, glucagon's effect is opposite that of insulin in this condition. However, hyperglucagonemia does not appear to have an effect on whole-body leucine flux (proteolysis) when this co-exists with maintained or "normal" insulin levels (20). Hence, the more favorable impact on body N-retention by a dietary mixture of carbohydrate and protein as compared with dietary protein alone might be due, in part, to these interactions between glucagon and insulin on amino acid utilization.

The activity of the sympathetic nervous system increases when carbohydrate (glucose) is consumed, but not when protein or fat are ingested (87, 88, 94). Hence, the effect of catecholamines on in vivo aspects of protein and amino acid metabolism is of potential importance. This point of view is supported by the suggestion made a number of years ago that epinephrine could reduce proteolysis (41), whereas an acceleration of proteolysis by catecholamines was earlier assumed, possibly because in physical trauma and infection, which lead to a protein catabolic state, there is a hyperepinephrinemia (91). To date, there are a limited number of studies that have reported findings about the relationships between epinephrine and dynamic aspects of amino acid metabolism. However, from the summary presented in Table 13 it is reasonable to draw the tentative conclusion that β-agonists reduce whole-body leucine oxidation, and possibly protein breakdown, whereas β-blockade increases leucine oxidation. If these alterations in plasma leucine kinetics reflect the global response of body amino acid metabolism, then a reasonable interpolation of the data leads to the conclusion that the overall effects of SNS activation are similar, but not necessarily identical to that of insulin. It is intriguing to speculate that the potentially greater nitrogen-sparing effect of carbohydrate relative to lipid is related to a cooperative or synergistic action of insulin and the sympathetic nervous system on amino acid metabolism.

Whether the glucocorticoids modulate the physiologic effects of energy and exogenous amino acids on nitrogen retention and sparing, as discussed above, is not yet clear. It has been appreciated for some time that administration of pharmacological amounts of corticoids in man causes muscle wasting (59). As summarized in Table 13, Gelfand et al. (42) did not find a change in leucine flux (protein breakdown) when four obese subjects received a cortisol infusion for 72 h, but three other studies involving glucocorticoid administration in normal subjects have reported increases in post-absorptive whole-body proteolysis (see Table 13) and, where measured, in leucine oxidation (8). However, there seems to be some disagreement concerning the extent to which glucocorticoids might continue with these same effects during the feeding period.

Alanine and glutamine fluxes are also increased by glucocorticoids (Table 13). However, the enhanced whole-body proteolysis due to glucocorticoid action may not be due to increased muscle (myofibrillar) protein breakdown, since urinary 3-N^{τ}-methylhistidine is apparently not affected (8). It is likely that the length, as well as dose, of corticoid administration is important in this context, at least this is the case in the rat (47, 48, 82).

Although the glucocorticoid effects noted above are exerted within a physiologic range of hormone levels, it is not yet possible to state whether

this counter-regulatory hormone can be implicated in the normal regulation of whole-body protein and amino acid metabolism or account, in mechanistic terms, for the nitrogen-sparing actions of changes in dietary energy and protein that we have covered so far. The same can be said for the involvement of growth hormone, which attenuates the effects of glucocorticoid (46) and the iodothyronines.

Role of alanine and glutamine

With reference to our preceding discussion, particularly in relation to the effects of glucose and hormones on muscle protein metabolism and amino acid balance, it is pertinent to recall that alanine and glutamine account for 60–80 % of the amino acids released from the skeletal musculature, with muscle serving as the major source of plasma alanine in post-absorptive human subjects (19). In view of the important roles played by these amino acids in inter-organ nitrogen transport, and because alanine is qualitatively the most important gluconeogenic amino acid and an important determinant of urea production (92), the N-sparing effects of energy sources on nitrogen balance might be exerted by a significant influence on the release of these amino acids from muscle and their subsequent fate in splanchnic nitrogen metabolism and hepatic gluconeogenesis.

Short-term infusion of glucose does suppress alanine release from forearm muscle (23), alanine infusion increases urea production (92) and large doses of oral glucose and intravenous glucose restrain alanine uptake by the splanchnic bed (28, 29). These responses of alanine metabolism are generally consistent with the decrease in loss of nitrogen from the whole body during long-term glucose infusion. However, glucose infusion has been shown to increase whole-body alanine flux (70), which suggests that tissues other than muscle, possibly the gut, account for an increased rate of alanine formation and entry into plasma. Nevertheless, the important point is that this effect does not correlate with the diminished rate of urea production (72). This is also the case when a carbohydrate-rich, low-protein diet is consumed (93). Hence, the role that the glucose-induced diminished output of muscle alanine plays in relation to the body nitrogen-sparing property of glucose remains unclear. More extensive and elegant studies on the fate of alanine nitrogen are now desirable to further clarify how glucose exerts its N-sparing effects and the stoichiometric relations between alanine metabolism and urea N-formation under different metabolic states.

In light of these findings for alanine, it might be that the regulation of glutamine release from skeletal muscle offers a further clue concerning the metabolic basis for the favorable effect of glucose administration on nitrogen retention. This latter amino acid accounts for more than 60 % of the free amino acid pool in skeletal muscle, and it serves as a precursor in biosynthetic processes and as an important vehicle for nitrogen and carbon transport between body tissues. Furthermore, it may have regulatory functions in relation to the synthesis and breakdown of proteins, and it serves as a gluconeogenic precursor. However, glucose infusion does not alter glutamine release from skeletal muscle (23), nor does the output

change after a protein meal (22). Thus, it does not appear that altered glutamine flux and interorgan flow is responsible for the N-sparing effect of glucose, at least in reference to its net output from the periphery and the consequences of short-term glucose administration. Thus, the role of glutamine, despite its known and major function in N-homeostasis, in relation to the energy-nitrogen relations with which we have been concerned remains to be clarified.

Lipids and amino acid kinetics

Briefly, with regard to the effect of lipid sources it was observed some years ago by Sherwin et al. (74) that an infusion of β-hydroxy-butyrate in non-obese and obese subjects reduced urinary N-excretion, and this has been confirmed (64). This effect of the ketone bodies is apparently attributable to a stimulation of amino-acid incorporation into proteins, accompanied by, or causally related to, a diminished amino acid oxidation (54, 62) which may be exerted at the level of the skeletal muscle. Of possible interest, however, is the finding that whole-body proteolysis does not seem to be affected by ketone bodies (54, 83), whereas triglyceride (LCT) and medium chain fatty acids appear to reduce both amino acid (leucine) oxidation, as well as protein turnover (7, 77). Thus, fatty acids and ketone bodies may exert their N-sparing effects via different mechanisms. This tentative conclusion is drawn, however, from a limited number of in vivo studies, and there is a need for further comparative investigations to better define the physiologic mechanisms underlying the effects of different lipid-energy sources on whole body, organ and interorgan N-metabolism.

Finally, in terms of attempting to define the underlying metabolic effects of mixtures of fuels on protein and amino acid metabolism it is of interest to note that the redox state of the cell might be an important mechanism responsible for differences in the pattern of gene expression (e.g., (1)) and, thus, protein synthesis and breakdown. If this turns out to be the case, then not only is the quantitative availability of glucose or lipid important, with implications for the generation of high-energy pyrophosphate bonds, but they also may exert effects on N-metabolism through oxidation/reduction mechanisms. This clearly would increase the complexity, but also the beauty of the eventual picture that comprehensively portrays the cellular and molecular events responsible for the metabolic interactions between protein and energy and their nutritional significance.

Summary and conclusion

The relations between energy and nitrogen (protein) have been reviewed and it is well documented that both the level and source of the dietary energy, as well as the nitrogen supply, have profound effects on body protein balance. Some of the underlying metabolic events responsible for the interactions between energy-yielding and nitrogen-containing substrates have been explored, as well as the impact of body protein turnover on energy expenditure. These various interrelations are dependent upon a complex of inter-related mechanisms, and it is clear that our understand-

ing of these mechanisms is still quite rudimentary, despite the practical and clinical importance of these aspects of metabolism and nutrition. A more complete series of investigations, including studies of dose responses, on these various aspects of protein-energy relations in both smaller and larger mammalian species would be highly desirable for reasons of basic physiologic interest and for the eventual improved practice of nutrition knowledge.

References

1. Abate C, Patel L, Rauscher FJ III, Curran T (1990) Science 249:1157–1161
2. Abbott WGH, Howard BV, Christin L, Freymond D, Lillioja S, Boyce VL, Anderson TE, Bogardus C, Ravussin E (1988) Am J Physiol 255:E332–E337
3. Arfvidsson B, Zachrisson H, Moller-Loswick A-C, Hyltander A, Sandstrom R, Lundholm K (1991) Am J Physiol 260:E46–E52
4. Baker DH, Becker DE, Norton HW, Jensen AH, Harmon BG (1966) J Nutr 88:391–396
5. Baker DH, Becker DE, Norton HW, Jensen AH, Harmon BG (1966) J Nutr 89:441–447
6. Baker JP, Detsky AS, Stewart S, Whitwell J, Marliss EB, Jeejeebhoy KN (1984) Gastroenterol 87:53–59
7. Beaufrere B, Tessari P, Cattalini M, Miles J, Haymond MW (1985) Am J Physiol 249:E175–E182
8. Beaufrere B, Horber FF, Schwenk WF, Marsh HM, Matthews D, Gerich JE, Haymond MW (1989) Am J Physiol 257:E712–E721
9. Bennet WM, Connacher AA, Scrimgeour CM, Smith K, Rennie MJ (1989) Clin Sci 76:447–454
10. Bennet WM, Connaher AA, Scrimgeour CM, Jung RT, Rennie MJ (1990) Am J Physiol 259:E185–E194
11. Black JL, Griffiths DA (1975) Brit J Nutr 33:399–412
12. Blaxter KL (1989) Energy metabolism in animals and man. Cambridge University Press, Cambridge, UK, 336 pages
13. Brody S (1945) Bioenergetics and growth, with special reference to the efficiency complex in domestic animals. Reinhold Publishing Corp., New York
14. Calloway DH, Spector H (1954) Am J Clin Nutr 2:405–412
15. Campbell RG (1988) Nutr Res Revs 1:233–253
16. Carr JR, Boorman KN, Cole DJA (1977) Brit J Nutr 37:143–155
17. Castellino P, Luzi L, Simonson DC, Haymond M, DeFronzo RA (1987) J Clin Invest 80:1784–1793
18. Cathcart EP (1909) J Physiol 39:311–330
19. Consoli A, Nurjhan N, Reilly JJ jr, Bier DM, Gerich JE (1990) Am J Physiol 259:E677–E684
20. Couet C, Fukagawa NK, Matthews DE, Bier DM, Young VR (1990) Am J Physiol 258:E78–E85
21. Darmaun D, Matthews DE, Bier DM (1988) Am J Physiol 255:E366–E373
22. Elia M, Livesey G (1983) Clin Sci 64:517–526
23. Elia M, Neale G, Livesey G (1985) Clin Sci 69:123–133
24. Elwyn DH (1980) Crit Care Med 8:9–20
25. Elwyn DH, Gump FE, Iles M, Long Cl, Kinney JM (1988) Metabolism 27:325–331
26. FAO/WHO (1973) Energy and protein requirements. Report of a Joint FAO/WHO Ad Hoc Expert Committee. WHO Technical Report Series, No. 522, World Health Organization, Geneva, Switzerland

27. FAO/WHO/UNU (1985) Energy and protein requirements. Report of a joint FAO/WHO/UNU Consultation. Tech Rep Ser No. 724, 1985 World Health Organization, Geneva, Switzerland
28. Felig P, Wahren J, Hendler R (1975) Diabetes 24:468–475
29. Ferrannini E, Wahren J, Felig P, DeFranzo RA (1980) Metabolism 29:28–35
30. Ferrannini E, Barrett EJ, Bevilacqua S, Jacob R, Walesky M, Sherwin RS, DeFronzo RA (1986) Am J Physiol 250:E686–E694
31. Flakoll PJ, Kulaylat M, Frexes-Steed M (1990) Am J Physiol 257:E839–E847
32. Folin O (1905) Am J Physiol 13:66–115
33. Folin O (1905) Am J Physiol 13:117–138
34. Frexes-Steed M, Warner ML, Bulus N, Flakoll P, Abumrad MN (1990) Am J Physiol 258:E907–E917
35. Fryburg DA, Barrett GJ, Louard RJ, Gelfand RA (1990) Am J Physiol 259:E477–E482
36. Fukagawa NK, Minaker KL, Rowe JW, Goodman MN, Matthews DE, Bier DM, Young VR (1985) J Clin Invest 76:2306–2311
37. Fukagawa NK, Minaker KL, Rowe JW, Matthews DE, Bier DM, Young VR (1988) Metabolism 37:371–377
38. Fukagawa NK, Minaker KL, Young VR, Matthews DE, Bier DM, Rowe JW (1989) Am J Physiol 256:E288–E294
39. Fuller MF, Weekes TEC, Cadenhead A, Bruce JB (1977) Brit J Nutr 38:489
40. Fuller MF, McWilliam R, Wang TC, Giles LR (1989) Brit J Nutr 62:255–267
41. Garber AJ, Karl JE, Kipnis DM (1976) J Biol Chem 251:851–857
42. Gelfand RA, Matthews DE, Bier DM, Sherwin RS (1984) J Clin Invest 74:2238–2248
43. Gelfand RA, Barrett EJ (1987) J Clin Invest 80:1–6
44. Gill M, France J, Summers M, McBridge BW, Milligan LP (1989) J Nutr 119:1287–1299
45. Hoerr RA, Matthews DE, Bier DM, Young VR (1991) Am J Physiol 260:E111–E117
46. Horber FF, Haymond MW (1990) J Clin Invest 86:265–272
47. Kayali AG, Young VR, Goodman MN (1986) Am J Physiol 252:E621–E626
48. Kayali AG, Goodman MN, Lin J, Young VR (1990) Am J Physiol 259:E699–E705
49. Kraenzlin ME, Keller V, Keller A, Thelin A, Arnaud MJ, Stauffacher W (1989) J Clin Invest 84:388–393
50. Krempf M, Hoerr RA, Pelletier VA, Marks LA, Gleason R, Young VR (1991) An isotopic study of the effect of dietary carbohydrate on splanchnic uptake of dietary leucine and phenylalanine (submitted for publication)
51. Krezowski PA, Nuttal FZ, Gannon MC, Bartosh NH (1986) Am J Clin Nutr 44:847–856
52. Lamont LS, Patel DG, Kalhan SS (1989) J Appl Physiol 67:221–225
53. Matthews DE, Pesola G, Campbell RG (1990) Am J Physiol 258:E948–E956
54. Miles JM, Nissen SL, Rizza RA, Gerich JE, Hayward WW (1983) Diabetes 32:197–205
55. Miles JM, Nissen SL, Gerich JE, Haymond MW (1984) Am J Physiol 247:E166–E172
56. Millward DJ, Rivers JPN (1988) Europ J Clin Nutr 42:367–393
57. Millward DJ, Price GM, Pacy PJH, Halliday D (1990) Proc Nutr Soc 49:473–487
58. Munro HN (1951) Physiol Rev 32:449–488
59. Munro HN (1964) In: Munro HN, Allison JB (ed) Mammalian Protein Metabolism. Academic Press, New York. Chpt 1, pp 381–481
60. Muramatsu T (1990) Nutr Res Revers 3:211–228
61. Nair KS, Halliday D, Matthews DE, Welle SL (1987) Am J Physiol 253:E208–E213

62. Nair KS, Welle SL, Halliday D, Campbell RG (1988) J Clin Invest 82:198–205
63. Nobukuni Y, Mitsubuchi H, Akaboshi I, Indo Y, Endo F, Yashioka A, Matsuda I (1991) J Clin Invest 87:1862–1866
64. Pawan GLS, Semple SJG (1983) Lancet i:15–17
65. Pineda O, Torun B, Viteri FE, Arroyave G (1981) In: Bodwell CE, Adkins JS, Hopkins DT (ed) Assessment and in vitro estimation. AVI Publ Co Inc, Westport, Connecticut, pp 29–42
66. Pozefsky T, Felig P, Tobin JD, Soeldner JS, Cahill GF (1969) J Clin Invest 48:2273–2282
67. Reeds PJ (1990) Proc Nutr Soc (Engl) 49:489–497
68. Reeds PJ, Harris CI (1981) In: Waterlow JC, Stephen JML (ed) Nitrogen Metabolism in Man. Applied Science Publishers, London and New Jersey, pp 391–408
69. Richardson DP, Wayler AH, Scrimshaw NS, Young VR (1979) Am J Clin Nutr 32:2217–2226
70. Robert J-J, Bier DM, Zhao XH, Matthews DE, Young VR (1982) Metabolism 31:1210–1218
71. Robert JJ, Cummins JC, Wolfe RR, Young VR (1982) Diabetes 31:203–211
72. Shangraw RE, Stuart CA, Prince MJ, Peters EJ, Wolfe RR (1988) Am J Physiol 255:E548–E558
73. Shaw JHF, Holdaway CM (1988) J Paren Enter Nutr 12:433–440
74. Sherwin RS, Hendler RG, Felig P (1975) J Clin Invest 55:1382–1390
75. Simmons PS, Miles JM, Gerich JE, Haymond MW (1984) J Clin Invest 73:412–420
76. Tessari P, Trevison R, Inchiostro S, Biolo G, Nosandini R, de Kreutzenberg SV, Duner E, Tiengo A, Crepaldi G (1986) Am J Physiol 251:E334–E342
77. Tessari P, Nissen SL, Miles JM, Haymond MW (1986) J Clin Invest 77:575–581
78. Tessari P, Inchiostro S, Biolo G, Trevisan R, Fantin G, Marescotti MC, Iori E, Tiengo A, Grepalde G (1987) J Clin Invest 79:1062–1069
79. Tessari P, Biolo G, Inchiostro S, Sacca L, Nosadini R, Boscarato T, Trevisan R, de Kreutzenberg SV, Tiento A (1990) Am J Physiol 259:E96–E103
80. Thompson GN, Bresson JL, Pacy PJ, Bonnefont JP, Walter JH, Leonard JV, Saudbray JM, Halliday D (1990) Am J Physiol 258:E654–E660
81. Thompson GN, Walter JH, Leonard JV, Halliday D (1990) Metabolism 39:799–807
82. Tomas FM, Munro HN, Young VR (1979) Biochem J 178:139–146
83. Umpleby AM, Chubb D, Boroujerdi MK, Sonksen PH (1988) Clin Sci 74:41–48
84. Vazquez JA, Morse EL, Adibi SA (1986) J Clin Invest 76:737–743
85. Waterlow JC (1984) Quart J Exptl Physiol 69:409–438
86. Waterlow JC, Millward DJ (1990) In: Wieser W, Gnaiger E (ed) Energy transformation in cells and organisms. Georg Thieme Verlag, Stuttgart, pp 277–282
87. Welle SL, Lilavivathana V, Campbell RG (1980) Metabolism 29:806–809
88. Welle SL, Lilavivathana V, Campbell RG (1981) Metabolism 30:953–958
89. Westphal SA, Gannon MC, Nuttall FQ (1990) Am J Clin Nutr 52:267–272
90. White RG, Hume ID, Nolan JV (1988) J Comp Physiol 158:237–246
91. Wilmore DW, Long JM, Mason MC, Skreen RW, Pruitt BA (1974) Ann Surg 180:653–669
92. Wolfe RR, Jahoor F, Shaw JHF (1987) J Parent Ent Nutr 11:109–111
93. Yang RD, Matthews DE, Bier DM, Wen Z-M, Young VR (1986) Am J Physiol 250:E39–E46
94. Young JB, Rowe JW, Pallotta JA, Sparrow D, Landsberg L (1980) Metabolism 29:532–539
95. Young VR, Robert JJ, Motil KJ, Matthews DE, Bier DM (1981) In: Waterlow JC, Stephen JML (eds) Nitrogen Metabolism in Man. Applied Science Publishers Ltd., Barking, Essex, England, pp 417–447

96. Young VR, Bier DM, Pellett PL (1989) Am J Clin Nutr 50:80–92
97. Young VR, Bier DM, Pellett PL (1990) Am J Clin Nutr 51:494–496
98. Young VR, Marchini JS (1990) Am J Clin Nutr 51:270–289
99. Young VR, Yu Y-M, Fukagawa NK (1992) In: Kinney JM (ed) Energy metabolism: Tissue Determinants and Cellular Corollaries. Raven Press, New York, pp 139–160 and 439–466

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