ORIGINAL RESEARCH

A Review of Issues of Dietary Protein Intake in Humans

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Considerable debate has taken place over the safety and validity of increased protein intakes for both weight control and muscle synthesis. The advice to consume diets high in protein by some health professionals, media and popular diet books is given despite a lack of scientific data on the safety of increasing protein consumption. The key issues are the rate at which the gastrointestinal tract can absorb amino acids from dietary proteins (1.3 to 10 g/h) and the liver's capacity to deaminate proteins and produce urea for excretion of excess nitrogen. The accepted level of protein requirement of $0.8 \text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ is based on structural requirements and ignores the use of protein for energy metabolism. High protein diets on the other hand advocate excessive levels of protein intake on the order of 200 to 400 g/d, which can equate to levels of approximately $5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, which may exceed the liver's capacity to convert excess nitrogen to urea. Dangers of excessive protein, defined as when protein constitutes > 35% of total energy intake, include hyperaminoacidemia, hyperammonemia, hyperinsulinemia nausea, diarrhea, and even death (the "rabbit starvation syndrome"). The three different measures of defining protein intake, which should be viewed together are: absolute intake (g/d), intake related to body weight ($\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$) and intake as a fraction of total energy (percent energy). A suggested maximum protein intake based on bodily needs, weight control evidence, and avoiding protein toxicity would be approximately of 25% of energy requirements at approximately 2 to $2.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, corresponding to 176 g protein per day for an 80 kg individual on a 12,000kJ/d diet. This is well below the theoretical maximum safe intake range for an 80 kg person (285 to 365 g/d).

Key Words: increased protein intake, amino acid absorption, urea synthesis, maximum protein intake, weight loss

Historical Background

Much controversy exists over the advantages and disadvantages of various quantities of protein consumption and the metabolic fate of the amino acid content of

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various proteins, mainly due to the limited amounts of data pertaining to protein metabolism and amino acid kinetics in humans. Two clearly separate areas of interest can be identified in regard to protein intake, the first being the current debate on the merits of increased protein intake at the expense of carbohydrates in relation to weight loss and diabetic glycemic control, the second being the long-standing interest of those in the health, fitness, and body building fraternity with increased protein intake for perceived benefits in muscle development.

A comprehensive study of dietary protein in weight loss and glucose homeostasis, focusing particularly on leucine metabolism has been published recently by Layman et al. (1). Essentially dietary protein requirement is described as the minimum level of protein necessary to maintain short-term nitrogen balance under conditions of controlled energy intake and is quantified as the Recommended Daily Allowance (RDA) in the US. This level assumes the primary use of amino acids as substrates for synthesis of body proteins; however there is mounting evidence that additional metabolic roles for some amino acids require plasma and intracellular levels above minimum needs for protein synthesis (1). Recently a meta-analysis of 235 non-athletic individuals gathered from 19 nitrogen balance studies for estimating protein requirements in healthy adults found the median estimated average requirement (EAR), and 97.5th percentile (RDA) to be 105 mgN · kg^{-1} · d^{-1}, and 132 mgN · kg^{-1} · d^{-1} respectively (2). This corresponds to 0.65 and 0.83 g good quality protein · kg^{-1} · d^{-1}, or 52 g and 66.4 g per day respectively for an 80 kg individual.

As protein foods are generally expensive and often associated with saturated fat, protein RDA guidelines set the minimum level needed to prevent deficiency. Combining US protein and fat recommendations (average total energy intake of 820 kcal/d) and the average US energy intake of approximately 2100 kcal/d (3), it is possible to estimate by default the carbohydrate intake current nutrition policy recommends at 1280 kcal/d (320 g/d), which produces a carbohydrate:protein intake ratio of > 3.5 (1). Although no minimum RDA has been established for carbohydrate intake, the minimum daily carbohydrate requirement for tissues, which are obligate users of glucose for energy, can be determined at approximately 100 to 200 g glucose/d (4), giving a dietary intake ratio of approximately 1.5 for the minimum metabolic needs for carbohydrate to protein. Thus current nutrition recommendations suggest a balance of macronutrients with minimum levels of protein and fat and elevated intake of carbohydrate (1). This is despite evidence that high carbohydrate diets may increase blood triglycerides (5), reduce fat oxidation (6), and reduce satiety (7).

Some evidence for an increased protein intake in the Western diet, however, is the possible reliance on protein as an energy source in the diet of our ancestors prior to the development of agriculture (8) as verified in the diet of contemporary hunter-gatherers (9). The main aspect of this proposal is that the phenotypic characteristics of modern humans evolved primarily over a 2 to 3 million year period during which hunted game made a progressively significant contribution to total energy intake (10). It has been estimated that the range of dietary protein energy intake for worldwide-hunter gatherers (19 to 35%) (9) would considerably exceed the mean intake levels found in Western diets in general (15.5%) (11) and in the Australian diet (17.1%) (12).

Modern day athletes or individuals undertaking physical training regimes are often conscious of increasing their protein intake, a characteristic of athletes elegantly described by food patterns of those competing in the XI Olympiad in Berlin (1936), where consumption levels of > 800 g of meat per day were reported (13). A comprehensive review of protein needs for strength and endurance trained athletes have been suggested at 1.4 to 1.8 g · kg^{-1} · d^{-1} and 1.2 to 1.4 g · kg^{-1} · d^{-1} respectively, corresponding to 112 to 144 and 96 to 112 grams protein per day for an 80 kg individual respectively (14). Evidence suggests however, that subgroups such as gym-goers, active people, and bodybuilders have felt that their protein needs exceed recommended levels, and are consuming in the area of 150 to 400 grams per day (15-17). High protein diets and popular "fad-diets" that claim to be "high protein, low carbohydrate," recommend intakes between 71 to 162 grams of protein per day (18-20) also fuel interest in increased consumption of protein. This is despite the fact that no studies have evaluated the upper limit of amino acid intake (21), and no formal risk assessment paradigm for intakes of amino acids that are in significant excess of physiological requirements have been established (22). This should be a concern for any health professional advocating a high protein diet.

Protein Metabolism

Advances in understanding protein metabolism have been made in the last few years with the advent of dual tracer methodology for assessing differences between exogenous and endogenous amino acid contribution to the protein pool, enhancing our comprehension of amino acid absorption kinetics. The application of radioactive and stable isotopes for the measurement of gluconeogenesis using mass isotopomer distribution analysis (MIDA) have also furthered our understanding the role of amino acids play postprandialy (23). However, the role of dietary protein and amino acids in modulating insulin and glucagon secretion are less clear, as is an understanding of what fraction of amino acid load contributes to structural/functional protein needs, oxidation, gluconeogenesis, or a combination of all three.

Although protein digestibility has been established for milk, pea, whey, casein, and free amino acids derived from enteral protein, less is known about specific absorption rates of protein-based foods such as meat, chicken, fish, and legumes. Secondly, there is a dearth of practical data on actual protein absorption rates measured in g/h or g · h^{-1} · kg^{-1}. The practical implications for understanding this information is exemplified by a novice bodybuilder who may consume 250 to 400 g of whey protein isolate on a daily basis, in the belief that it will promote greater skeletal muscle anabolism, a debatable point at best (24, 25); however, a more important issue is how does the human body deal with these large (> 200 g/d) amounts of protein?

To develop a better understanding of amino acid kinetics, the initial part of this article will examine protein metabolism, focusing on the quantification of maximal protein consumption using available data on maximal rates of urea synthesis, and amino acid absorption rates and suggest using this data, a possible upper limit (measured in grams per day) for the rate of amino acid metabolism. The article will then outline some of the complex interplay of hormonal regulation of insulin and glucagon by specific amino acids.

Maximal Rate of Urea Synthesis and Excretion

Amino acid catabolism must occur in a way that does not elevate blood ammonia (26). Catabolism of amino acids occurs in the liver, which contains the urea cycle (26), however the rate of conversion of amino acid derived ammonia to urea is limited. Rudman et al. (27) found that the maximal rate of urea excretion (MRUE) in healthy individuals was 55 mg urea $N \cdot h^{-1} \cdot kg^{-0.75}$, which is reached at an intake level of 0.53 g protein $N/kg^{-0.75}$ At higher protein intakes there is no further increase in urea excretion rate, but a prolongation of the duration of MRUE, often in excess of 24 h (27).

In a further investigation of the fate of protein nitrogen, Rudman et al. (27) were able to quantify the temporary accumulation of urea in body water during MRUE and the amount hydrolyzed in the gastrointestinal tract. Subsequently an algorithm was developed to estimate the capacity of the liver to deaminate amino acids and produce urea, termed the maximal rate of urea synthesis (MRUS). In a study on 10 healthy subjects, the MRUS averaged 65 mg urea $N \cdot h^{-1} \cdot kg^{-0.75}$ (with a range of 55 to 76). Thus the level of dietary protein that can be deaminated and processed through to urea by the liver in a 24-h period is dependent on body weight and individual variation in efficiency of the process, as indicated in Table 1. An 80 kg individual, for instance, could deaminate up to 301 g protein per day, but may be limited to 221 g protein per day, given the range in MRUS determined by Rudman et al. (27). However, the safe intake level of protein consumption may even be slightly higher than these figures, as not all protein is deaminated and converted to urea. A certain amount of protein as indicated by the RDA is used directly for structural/functional purposes, including bone and soft tissue growth, maintenance and repair plus production of hormones, antibodies, and enzymes, thus not requiring deamination.

The US recommended dietary allowance (RDA) of 0.8 g $\cdot kg^{-1} \cdot d^{-1}$ (28), set this level of necessary protein intake for structural requirements to cover the needs of 97.5% of the population. Adding this protein requirement (64 g/d for an 80 kg individual) to the level of protein that can be converted to urea, yields a *theoretical* maximal daily protein intake based on body weight and efficiency of urea synthesis in individuals. An 80 kg individual, for example, could theoretically tolerate 325 g protein per day (range 285 to 365 g) without showing symptoms of hyperammonemia and hyperaminoacidemia. Such levels are certainly not advocated by the authors and no practical rationale exists for such elevated protein intakes. In fact, common sense would even dictate that we accept the lower end of the range as the maximum safe intake levels, to allow for individuals with reduced MRUS. Hence, for an 80 kg individual, 285 g protein per day should be viewed as an absolute maximum. Even this amount would be equivalent to the consumption of approximately 1 kg of lean meat per day. A more realistic intake of approximately half this amount would contribute approximately 60 g protein to structural needs and a further approximately 80 g to bodily energy needs, either directly, through gluconeogenesis or as stored fat. The advantage being that this protein could displace fat or carbohydrate from the diet, increase satiety (plus yield less energy due to its higher rate of thermogenesis) thus helping in weight control, a controversial but promising area of research and will be discussed later. It may be prudent to point out, however, that the given range of MRUS determined by Rudman et al. (27),

had significant limitations, as it consisted of a small sample size (10 normal and 34 cirrhotic subjects), excluded individual differences according to age, sex, previous diet history, training status, and was undertaken more than 30 y ago.

The dangers of excessive protein intake should not be underestimated and have been recognized historically through the excess consumption of lean wild meat by early American explorers leading to a condition referred to as "rabbit starvation syndrome," in which symptoms included nausea and diarrhea followed by death within 2 to 3 wk (29). This syndrome was explained as the inability of the human liver to sufficiently upregulate urea synthesis to meet "large" loads of protein (29). Some studies have shown animals can adapt to a high protein diet by upregulating amino acid metabolizing enzymes such as alanine and aspartate aminotransferases, glutamate dehydrogenase, and argininosuccinate synthetase (30), and increase mitochondrial glutamine hydrolysis in hepatocytes (31). Other studies, however, show that when animals are faced with large protein loads, the rate of gastric emptying is reduced as the catabolic and anabolic systems of the body become saturated, unable to deal with an excess of dietary nitrogen under acute conditions (32). This reduction in rate of gastric emptying subsequent to an elevated dietary protein intake suggests the presence of regulation at the gastric step to ensure the catabolic capacities of the liver are not exceeded (33). This negative feedback on stomach emptying rate and food intake could be affiliated with chemical, biochemical, and/or physical signals translated by the vagus nerve (34, 35). To what degree do these processes transpire in humans, and at what threshold intake of protein is currently unknown as very little data have been collected on humans consuming high protein diets for prolonged periods of time. The one well known case is that of the early 20th century Arctic explorer Vilhjalmur Stefansson, who after many years living with the Arctic Inuit and consuming a diet estimated to be approximately 50% energy as protein, returned to civilization and conducted a year-long experiment on himself at Bellevue Hospital in New York. During this time, Stefansson, a fit 72.5 kg man, consumed meat only, with a variable protein:fat ratio. During the first 3 d he became ill with the symptoms of "rabbit starvation" at a protein intake of 264 g/d, which was 45.3% of his energy intake (36). As the protein level was lowered slightly and replaced with extra fat, however, on the fourth and fifth days symptoms disappeared. This level of protein intake may have been sustainable if hepatic enzymes were given time to upregulate (as there were several years between Stefansson living with the Inuit and the experiment at Bellevue Hospital and any previous upregulation of hepatic enzymes would have diminished), but the result for this limited study (where $n = 1$) indicates that the values shown in Table 1 are at least realistic, although we would speculate that individuals would tend to be at the lower end of the range for MRUS without a lead-in time for upregulating hepatic enzyme function.

Protein Absorption Rates in Humans

Another critical aspect of protein metabolism involves the extent and rate of intestinal absorption of dietary protein. A limited number of protein studies investigating absorption rates of amino acid from specific protein sources such as casein, whey, milk, pea, egg, soy, and meat have been conducted. The metabolism of dietary

Table 1 Range of Daily Protein Intakes Based on Body Weight and the Algorithm for Maximal Rate of Urea Synthesis (MRUS) Developed by Rudman et al. (27) and Allowing for Protein Requirements Set by the RDA Where Deamination is Not Occurring

MRUS	Body weight (kg)											
mg N · h^{-1} · kg · $^{0.75}$	10	20	30	40	50	60	70	80	90	100	110	
	Daily protein maximal intakes based on MRUS (g)											
55	46	78	106	131	155	178	200	221	241	261	280	lower
60	51	85	115	143	169	194	218	241	263	285	306	
65	**55**	**92**	**125**	**155**	**183**	**210**	**236**	**261**	**285**	**308**	**331**	**mean**
70	59	99	135	167	197	226	254	281	307	332	357	
75	63	106	144	179	212	243	272	301	329	356	382	upper
	Daily protein intake based on RDA for structural use (g)											
	8	16	24	32	40	48	56	64	72	80	88	
	Maximum daily protein intake levels (g)											
55	54	94	130	163	195	226	256	285	313	341	368	lower
60	59	101	139	175	209	242	274	305	335	365	394	
65	**63**	**108**	**149**	**187**	**223**	**258**	**292**	**325**	**357**	**388**	**419**	**mean**
70	67	115	159	199	237	274	310	345	379	412	445	
75	71	122	168	211	252	291	328	365	401	436	470	upper

protein and amino acids is influenced by the composition of the specific protein, meal composition, timing of ingestion, and the amount or dose of the protein or amino acids ingested (37). The speed of absorption by the gut of amino acids derived from dietary proteins can also modulate whole body protein synthesis, breakdown, and oxidation (38, 39). Quantifying specific absorption rates of dietary amino acids from the gut in humans at a variety of doses is difficult due to the lack of specific data. A comprehensive analysis of existing data is difficult as many of the studies that provide sound methodology to study actual amino acid kinetics do not employ sizeable doses of amino acids and further, fail to provide data on the body mass of the subjects used. Interpreting results yields a crude but sufficient starting point for describing amino acid absorption from the gut, in a g/h absorption rate, rather than a more accurate g · h^{-1} · kg^{-1} measure.

Milk Proteins

Using [^{15}N]-labeling dietary protein methodology, 25 subjects (with mean BMI of 22.4 ± 2.5 kg/m^2) swallowed an ileal tube and ingested 30 g of [^{15}N]-milk protein (P) alone (295 mmol N), or supplemented with either milk fat (PF) (43 g of milk fat from 36 g butter and 46 g cream) or 100 g of sucrose (PS). In the 8-h period

after meal ingestion, the amount of dietary nitrogen recovered in the ileum via blood sampling in the forearm vein was 279.6 ± 1.3 mmol in the (P) group, 279.2 ± 1.2 mmol in (PS), and 278.1 ± 2.4 mmol in the (PF) group. This shows the true digestibility of exogenous milk protein nitrogen to be of the order of 94.6% with an average rate of protein absorption of 3.5 g/h (40).

Pea Protein

The gastrointestinal absorption of pea protein of 7 adults (4 males and 3 females with mean mass of 64 kg, ranging from 46 to 77 kg) was determined by ingesting 21.45 g (195 mmol N) of [^{15}N]-labeled pea protein. Total absorption was estimated at $89.4 \pm 1.1\%$, resulting in 19.2 g being absorbed in the 8-h postprandial period at a rate of 2.4 g/h (41). Another study investigated the ingestion of 30 g of raw purified pea protein either as [^{15}N]-globulins, (G meal) (301 mmol N) or as a mix of [^{15}N]-globulins and [^{15}N]-albumins, (GA meal) (22 g of pea globulins and 8 g of pea albumins, 299 mmol N). The ileal digestibility was $94.0 \pm 2.5\%$ and $89.9 \pm 4.0\%$ for the G and GA meals respectively yielding amino acid absorption rates of approximately 3.5 g/h and 3.4 g/h (42).

Egg Protein

The absorption of 25 g of ^{13}C-, ^{15}N-, and ^{2}H-labeled egg protein, both cooked (C) and raw (R) were evaluated. Measurements of mean $^{13}CO_2$ exhalation rate in breath after the ingestion continued for 6 h. The cumulative amount of administered dose of ^{13}C recovered in breath over the 6-h period was 17.23 ± 0.69 g (68.92%) for (C) and 8.20 ± 0.94 g (32.8%) for (R), giving an estimated absorption rate of 2.9 g/h and 1.4 g/h respectively for cooked and raw egg proteins (43).

Soy Protein Isolate (SPI)

Soy protein is believed to have a high nutritional quality for humans (44). The absorption rates of 30 g [316 mmol N] of [^{15}N]- soy protein isolate (SPI)] mixed with 100 g of sucrose and water were analyzed in subjects who had a mean body mass of 65 ± 9 kg (45). The overall true oro-ileal digestibility of SPI was $90.9 \pm 2.2\%$, at an absorption rate of 3.9 g/h, which is consistent with other studies of SPI absorption (46).

Tenderloin Pork Steak

Amino acid absorption from pork steak was determined crudely by comparison with intravenous infusions of varying amounts of mixed amino acid solution (47). A mixed amino acid solution (MAA) was designed to mimic that of the amino acid profile of a 200 g portion of tenderloin pork steak meal (PS), containing 36 g of protein and 20 g fat. The postprandial plasma amino acid profile of the subjects consuming the PS was measured and compared with the postprandial plasma amino acid profile of the intravenous infusions of the MAA solutions, which were infused at 6, 10, and 14 g/h on a separate day. The closest matching infusion rate of amino acids, which matched the amino acid pattern of the pork steak was 10 g/h ($r = 0.89$, $P < 0.001$). If pork protein is absorbed at 10 g/h then 36 g would be absorbed in

3.6 h. Interpretations of these findings however are difficult, as the study does not incorporate dual tracer methodology, allowing discrimination between endogenous and exogenous amino acid rate appearances.

Casein and Whey Protein

Absorption rates of "fast" and "slow" dietary proteins, whey (WP), and casein (CAS) respectively, provide an interesting contrast in protein absorption kinetics. In a study by Boirie et al. (38), subjects were fed 30 g (336 mmol N) of labeled whey protein (^{13}C-WP), or 43 g (479 mmol N) of labeled casein protein (^{13}C-CAS), with the same amount of dietary leucine (380 μmol/kg), where postprandial leucine balance is used as an index of protein deposition (39, 48). The rapid absorption of WP in the first 3 to 4 h accounted for the vast majority of amino acid absorption in the order of 8 to 10 g/h and ~ 6.1 g/h for CAS. A second study involved the repeated consumption of 2.3 g of whey protein every 20 min (RPT-WP), (a rate of ~ 7g/h), to mimic slowly absorbed amino acids. This was compared to a 30 g protein meal of amino acids (AA). Estimated absorption rates were ~ 8 g/h and 6 g/h for AA and RPT-WP, respectively (39).

Maximum Absorption Rates of Amino Acids

Absorption rates of amino acids estimated in this review (summarized in Table 2) are crude, yet serve as sufficient approximates given the absence of direct data pertaining specifically to amino acid absorption (i.e., grams per hour per kilogram of body weight). The absorption rate (measured as g/h), of free amino acids (AA), casein isolate (CAS), and whey protein isolate (WP) were greater than that of raw and cooked egg white, pea flour, and slightly greater than milk protein. Free amino acids with the same amino acid profile as casein protein elicits a fast transient peak of plasma amino acids, while casein releases amino acids slowly over many hours after consumption. This is consistent with other studies that show free amino acid mixtures induce a more rapid absorption than intact proteins (49, 50). The two milk protein fractions, micellar casein and the soluble whey protein have been synonymous with the concept of "slow," and "fast" digestibility of protein. A detailed discussion of these two milk protein fractions is

Table 2   Approximations of Amino Acid Absorption from Different Protein Sources

Protein source	Absorption rate (g/h)	Reference
Egg protein raw	1.3	43
Pea flour	2.4	41
Egg protein cooked	2.8	43
Pea flour: globulins & albumins	3.4	42
Milk protein	3.5	40
Soy protein isolate	3.9	46
Free AA	4.3	39
Casein isolate	6.1	38
Free AA (same profile as casein)	7-7.5	39
Whey isolate	8-10	38

beyond the scope of this article and provided elsewhere (51). It is however worth mentioning that WP is soluble, allowing faster gastric emptying, whereas casein clots in the stomach delaying gastric emptying, resulting in a slower release of amino acids (52).

An important question then must be posed: "Does a more rapidly absorbable protein result in greater *in vivo* protein synthesis?" This is a central issue of large protein consumption with fitness enthusiasts, athletes, and bodybuilders.

Early findings suggest that rapidly absorbed proteins such as free amino acids and WP, transiently and moderately *inhibit protein breakdown* (39, 53), yet stimulate protein synthesis by 68% [using nonoxidative leucine disposal (NOLD) as an index of protein synthesis] (54). Casein protein has been shown to inhibit protein breakdown by 30% for a 7-h postprandial period, and only slightly increase protein synthesis (38, 54). Rapidly absorbed amino acids despite stimulating greater protein synthesis, also stimulate greater amino acid oxidation, and hence results in a lower net protein gain, than slowly absorbed protein (54). Leucine balance, a measurable endpoint for protein balance, is indicated in Figure 1, which shows slowly absorbed amino acids (~ 6 to 7 g/h), such as CAS and 2.3 g of WP repeatedly taken orally every 20 min (RPT-WP), provide significantly better protein balance than rapidly absorbed amino acids (39, 54).
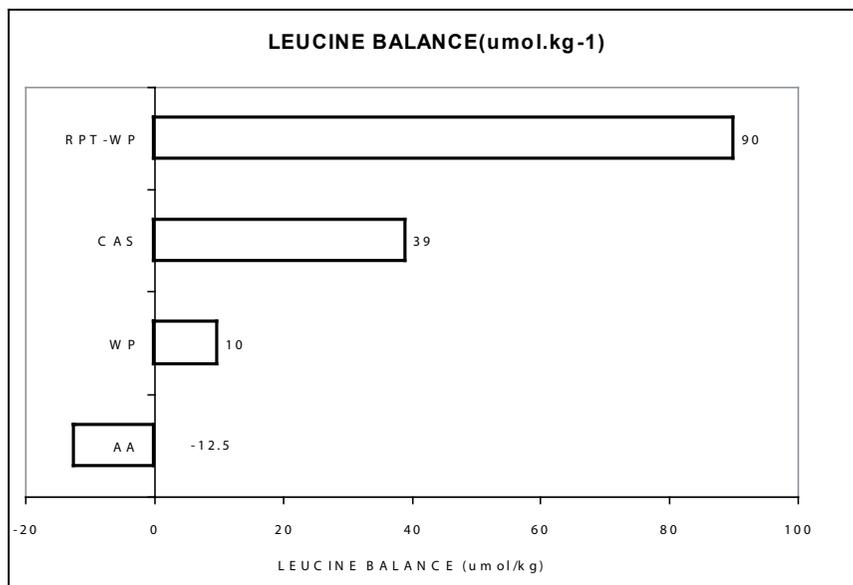

Figure 1—Leucine balance (a measurable endpoint for protein balance) as determined from rapidly absorbed protein; amino acids (AA) and whey protein (WP), compared to slowly digestible proteins; casein (CAS), and small doses of whey protein (RPT-WP 6.9 g/h) (adapted from 39). The misconception in the fitness and sports industries is that rapidly absorbed protein, such as WP and AA promote better protein anabolism. As the graph shows, slowly absorbed protein such as CAS and small amounts of WP (RPT-WP) provide four and nine times more protein synthesis than WP.

    This "slow" and "fast" protein concept provides some clearer evidence that although human physiology may allow for rapid and increased absorption rate of amino acids, as in the case of WP (8 to 10 g/h), this fast absorption is not strongly correlated with a "maximal protein balance," as incorrectly interpreted by fitness enthusiasts, athletes, and bodybuilders. Using the findings of amino acid absorption rates shown in Table 2 (using leucine balance as a measurable endpoint for protein balance), a maximal amino acid intake measured by the inhibition of proteolysis and increase in postprandial protein gain, may only be ~ 6 to 7 g/h (as described by RPT-WP, and casein) (38), which corresponds to a *maximal* protein intake of 144 to 168 g/d.

    The rate of amino acid absorption from protein is quite slow (~ 5 to 8 g/h, from Table 2) when compared to that of other macronutrients, with fatty acids at ~ 0.175 g $\cdot$ kg^{-1} $\cdot$ h^{-1} (~ 14 g/h) (55) and glucose 60 to 100 g/h (0.8 to 1.2 g carbohydrate $\cdot$ kg^{-1} $\cdot$ h^{-1}) for an 80 kg individual (56). From our earlier calculations elucidating the maximal amounts of protein intake from MRUS, an 80 kg subject could theoretically tolerate up to 301 to 365 g of protein per day, but this would require an absorption rate of 12.5 to 15 g/h, an unlikely level given the results of the studies reported above. However, some support for this level of absorption of amino acids is found when amino acids are infused intravenously at 50, 100, 150 and 250 mg $\cdot$ kg^{-1} $\cdot$ h^{-1} (57). This protocol investigated the relationship between the rate of infusion of amino acids and the muscle protein synthetic rate, which peaked at 150 mg $\cdot$ kg^{-1} $\cdot$ h^{-1}, corresponding to an absorption rate of 12 g/h for an 80 kg individual (57).

Amino Acid Regulation of Endocrine Hormones

Protein meals with their associated amino acid loads are known to stimulate the release of the pancreatic hormones glucagon and insulin into the circulatory system (47, 58).

Glucagon

Studies evaluating the response of glucagon to real foods have shown that a 200 g pork steak containing 36 g of protein stimulated a glucagon release, raising plasma levels from 180 ± 24 to 960 ± 115 ng/L after 120 min (47). Following binding to hepatic receptors, glucagon stimulates the enzyme adenylate cyclase on the membranes inner surface which catalyses the production of cyclic AMP (cAMP) (59), which in turn sets off a cascade of reactions resulting in the breakdown of glycogen to glucose (59). The major purpose of this aminogenic glucagon release is to stimulate hepatic glucose release in a bid to avert hypoglycemia resulting from the concomitant secretion of insulin (60).

    The level of glucagon release depends on the ratio of protein:carbohydrate content of a meal (61), (resulting in stimulation when the ratio is high, and suppression when the ratio is low) and the predominance of specific amino acids in a meal. Predominately glucagon-stimulating amino acids are serine, aspartate, glycine, asparagines, and phenylalanine (47). In one of the long-term studies (6 months) of elevated protein intakes, using whole food, subjects consuming 1.87 ± 0.26 g $\cdot$

$kg^{-1} \cdot d^{-1}$ of dietary protein, had a fasting plasma glucagon 34% higher than subjects consuming 0.74 ± 0.08 g $\cdot$ $kg^{-1} \cdot d^{-1}$ (62). Although not fully understood, glucagon is also involved in the disposal of amino acids after protein ingestion (63), particularly the increased hepatic uptake of glucongenic amino acids presumably for gluconeogenesis (60).

Insulin

The role of insulin in amino acid kinetics has not been fully elucidated and has been described as "the puzzling role of insulin" (64). It has been reported in the scientific literature however for well over 30 y that ingestion of carbohydrate-free protein meals such as beef and casein can promote a prompt and substantial rise in plasma insulin (65). In a study by Linn et al. (62) subjects fed a relatively high protein diet of 1.87 ± 0.26 g $\cdot$ $kg^{-1} \cdot d^{-1}$ over a 6-month period, consistently had elevated plasma insulin levels 8 h after the last protein meal.

In a study by Calbert et al. (24) pea protein hydrolysate (PPH), milk protein solution (MP), and whey protein hydrolysates (WP) were co-ingested with 15 g of glucose and compared to the hormonal pattern of 15 g of glucose solution alone. Despite similar glucose contents, peak insulin concentrations (occurring at the 20th minute) were two and four times higher after ingestion of both PPH and WP than after MP and glucose solutions respectively (24). Similar results supporting the insulinotropic properties of amino acids when added to carbohydrate have been obtained when comparing milk solutions with milk and sucrose together (40), while others have observed increased plasma insulin by as much as 100% above basal when using various combinations of insulinotrophic amino acids (56, 66-68).

Recently an insulin index of foods has been established which unexpectedly demonstrates that 1000 kJ of fish protein (~ 60 g) elicits a greater peak insulin level than 1000 kJ of white pasta (~ 60 g) (69). As previously mentioned in this review, amino acids, through stimulation of glucagon, release hepatic glucose. The hepatic glucose to insulin ratio of common foods, as shown in Figure 2, indicates either a significant insulin response to relatively small hepatic glucose release for meat and fish, (69) or a direct stimulatory effect of some amino acids on insulin release. The amino acids from beef augment an insulin response 1583 times that of its simultaneous hepatic glucose release via glucagon. Amino acids derived from fish result in a surge of insulin 775 times the magnitude of glucose stimulated release (69).

Not all studies however show the same large stimulatory effects of insulin by amino acids (40). It appears that before any elevation in plasma insulin can be detected, the plasma concentrations of amino acids must attain an as yet unidentified threshold level (24). For example, repeated doses of rapidly absorbed whey protein administered orally at 2.3 g every 20 min (6.9 g/h), stimulated mild hyperaminoacidemia, with no detectable rise in plasma insulin, while WP administered as a 30 g doses resulted in moderate increases in plasma insulin concentrations (39). Other factors also affect the degree of insulin release and need to be considered such as the amino acid make up of the ingested protein. Arginine, lysine, phenylalanine, ornithine, alanine, leucine, isoleucine, stimulate insulin, (56, 66) while the quantity of branched chain amino acid content in a meal, which are metabolized in muscle, also warrants consideration (70).
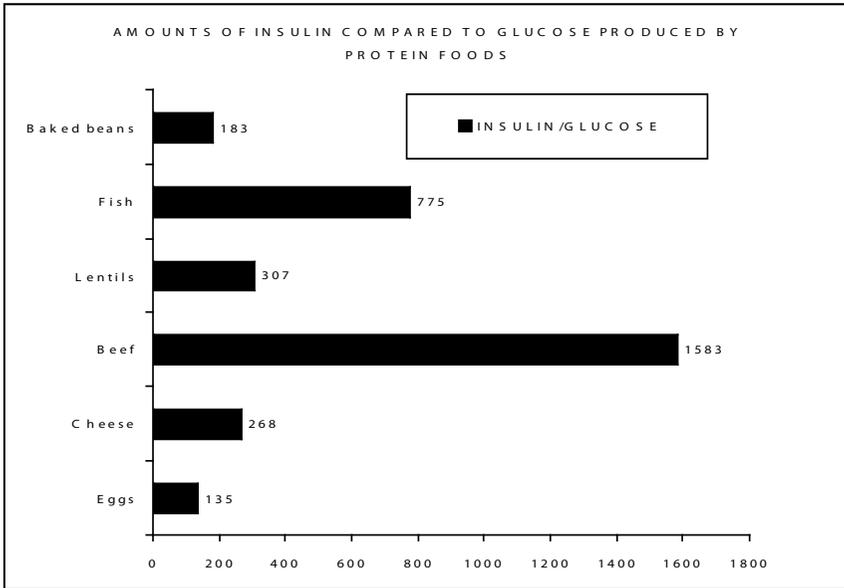
Figure 2—Insulin to glucose stimulated by protein based foods [adapted from (69)].

Although the study of the glucose/insulin relationship has been widely investigated, little data exists on the relationship between amino acids and insulin, and its relevance to the etiology of diabetes, disease, and health.

The Fate of Postprandial Amino Acids

Gluconeogenesis

The major fate of dietary amino acids in the Western diet appears to be gluconeogenesis (26, 71) and has been recently estimated to account for up to 60% of endogenous glucose production (23), while others estimate 47 to 60% (72-74). In one study a relatively high protein diet, $(1.87 \pm 0.26 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1})$, was shown to elevate gluconeogenesis by 40% (62). Thus gluconeogenesis should be viewed as a normal prandial process, not one limited to fasting periods (71), the alternative being the diversion of amino acid carbon to triglyceride production, a process which likely outcompetes gluconeogenesis only when carbohydrate intake is high (75).

Amino acids make up the major source of fuel for the liver and their oxidative conversion to glucose makes up approximately half of the daily oxygen consumption of the liver (71). The advantage of oxidizing most amino acids in the liver to glucose is that only the liver need expend the energy needed to synthesize the entire complex array of enzymes involved in amino acid oxidation. In this way all parts of the body can use energy derived from protein without the need for amino acid catabolizing enzymes (71). Complete oxidation of amino acids to CO_2 by the liver

does not occur as the ATP produced would be far more than the liver could use and the oxygen consumption greater than that available. Hepatic O_2 consumption has been estimated at 3000 mmoles/d (76). An intake of 110 g of animal protein per day contains ~ 1000 mmoles of amino acids, of which 10% is metabolized in extra-hepatic tissues, resulting in ~ 900 mmoles to be dealt with by the liver on a daily basis. To completely metabolize 900 mmoles of amino acid (~ 100 g of protein) to CO_2 the liver requires 3700 mmoles of oxygen, which accordingly is a physiological impossibility as the liver has to metabolize numerous other substances. However only 1420 mmoles of oxygen are needed to convert this amount of amino acids to glucose (70, 71), which can then be circulated for use in other body cells.

If diets relatively high in protein are to be tolerated it is likely that an evolutionary mechanism exists to metabolize some amino acids in peripheral tissues. Branched chain amino acids (BCAA) such as leucine, valine, and isoleucine, which yield high levels of ATP relative to their yield of glucose, are excellent candidates for this and are known to be oxidized in muscle tissue (71). Degradation of BCAA in muscle tissue is linked to the production of alanine and glutamine and the maintenance of glucose homeostasis (77). It has also been suggested that the glucose-alanine cycle accounts for > 40% of endogenous glucose production during exercise (77). Interestingly, as pointed out by Layman et al. (1), increased concentrations of leucine have the potential to stimulate muscle synthesis during catabolic conditions associated with food restriction (78) or exhaustive exercise (79).

Anaplerosis, Cataplerosis, and Amino Acid Metabolism in the Athlete

The consumption of large amounts of protein by athletes and bodybuilders is not a new practice (13). Recent evidence suggests that increased protein intakes for endurance and strength-trained athletes can increase strength and recovery from exercise (14, 80, 81). In healthy adult men consuming small frequent meals providing protein at $2.5 \text{ g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$, there was a decreased protein breakdown, and increased protein synthesis of up to 63%, compared with intakes of $1\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ (16). Subjects receiving $1\text{g} \cdot \text{kg}^{-1} \cdot \text{d}^{-1}$ underwent muscle protein breakdown with less evident changes in muscle protein synthesis. Some evidence suggests, however, that a high protein diet increases leucine oxidation (82, 83), while other data demonstrate that the slower digestion rate of protein (38, 54), and the timing of protein ingestion (with resistance training) (84) promote muscle protein synthesis.

One important role of dietary carbohydrate (through pyruvate) is in anaplerosis, the replenishing of Krebs cycle intermediates, (or tricarboxylic acid cycle intermediates—TCAI). The primary role of this cycle is to generate reduced forms of the enzymes NADH and $FADH_2$, transferring high energy electrons to the mitochondrial electron transport chain for use in the resynthesis of ATP (85). Five of the intermediates of Krebs cycle are involved in additional reactions which involve amino acids and will be limited if insufficient carbohydrate is available. Oxaloacetate and α-ketoglutarate are used in the synthesis of several amino acids such as phosphoenolpyruvate. Heme synthesis uses succinyl CoA, glutamine synthesis draws upon α-ketoglutarate, and citrate is the source of acetyl-CoA in the cystol and is used for the synthesis of lipids and amino acids (59, 70). Adequate dietary

carbohydrate during exercise is thus critical, because its availability is inversely related to the rate of exercise protein catabolism (86), hence adequate carbohydrate can prevent cataplerosis, the reverse of anaplerosis, which takes place in the absence of sufficient pyruvate (from carbohydrate). Gluconeogenesis can be considered cataplerotic and can result in a "drain" of Krebs cycle intermediates (70), which may result in a decreased production of ATP, and an increased muscle protein breakdown. There may be a critical minimum intake of carbohydrate to provide a sufficient flux of pyruvate to maintain anaplerosis (87), and prevent muscle protein breakdown via gluconeogenesis.

This has practical significance to fitness enthusiasts, athletes, and bodybuilders where 150 to 400 g of protein can be consumed per day (15-17), especially if consumed at the expense of sufficient carbohydrate. In elite athletes it has been clearly established that low glycogen availability for exercising skeletal muscles leads to fatigue more rapidly in prolonged exercise (88, 89). Other studies show the time until the onset of fatigue during high-intensity exercise in untrained individuals consuming diets deficient in carbohydrate is shortened (90-93), however similar results are not found in trained individuals (94). In high-intensity resistance training, fatigue may also be associated with carbohydrate depletion (95). While high protein diets have focused on protein and its value in building lean muscle and preventing protein breakdown, it is vitally important for athletes to understand that high protein consumption at the expense of sufficient amounts of carbohydrate can be potentially detrimental to lean muscle.

Dietary Advantages of Increased Protein Intake

As protein has a greater thermic effect than either fat or carbohydrate (96, 97) and a greater satiety value than fat or carbohydrate (98) there is strong circumstantial evidence for increased dietary protein as an effective weight loss strategy (99). Some clinical trials have shown that energy restricted elevated protein diets are more effective than high carbohydrate energy restricted diets for weight loss in overweight subjects (100, 101). Recently, low energy, isoenergetic diets (7100 kJ) containing either $1.6\ g \cdot kg^{-1} \cdot d^{-1}$ protein, carbohydrate < 40% of energy (HP), or $0.8\ g \cdot kg^{-1} \cdot d^{-1}$ protein and carbohydrates > 55% of energy (HC), yielded significant weight loss of 7.53 ± 1.44 kg and 6.96 ± 1.36 kg, respectively. The protein group however, lost more body fat and less lean body mass than the carbohydrate group (102). Suggestions made by Layman et al. (102) for these changes were 1) the lower energy efficiency of the protein diet, 2) lower insulin response with reduced carbohydrate, and 3) muscle protein sparing effect, of the protein or leucine specifically. Another study investigating the protein to carbohydrate ratio on body composition analyzed a high protein diet (HP) consisting of 27% protein, 44% carbohydrate, 29% fat as energy, and a standard protein diet (SP) consisting of 16% protein, 57% carbohydrate, 27% fat (103). Although weight loss (7.9 ± 0.5 kg) and total fat loss (6.9 ± 0.4 kg) did not differ between diet groups, total lean mass was significantly better preserved with the HP diet in women. Further, when a high protein diet (HP) consisting of 28% protein, 42% carbohydrate, 28% fat as energy was compared to a low protein diet (LP) 16% protein, 55% carbohydrate, 26% fat, overall weight loss was 5.2 ± 1.8 kg independent of diet composition (104). Women on the HP

diet, however, lost significantly more total (5.3 vs. 2.8 kg) and abdominal (1.3 vs. 0.7 kg) fat compared to woman on the LP diet. Collectively this data describes how elevating dietary protein intake may have a positive effect on preserving lean muscle, while lowering body fat content.

Defining Protein Intake

A confusing point in discussing dietary protein intake is the manner in which it is defined. What seems to be a high protein intake by one definition can appear quite moderate when represented in an alternative manner. There are three principal ways in which protein intake can be quoted: 1) as the absolute amount consumed in grams per day, 2) as a percentage contribution to daily energy intake based on its energy content of 17 kJ/g, and 3) as the amount consumed per kilogram of body weight per day (Table 3). An individual consuming a diet containing 35% energy as protein appears to be consuming a dangerously excessive level of protein. However, if total dietary energy intake is 8000 kJ/d, this equates to 165 g protein per day. For an 80 kg person this would be equivalent to ($2.1 \; g \cdot kg^{-1} \cdot d^{-1}$), well below the maximal level. Even for a 60 kg individual ($2.7 \; g \cdot kg^{-1} \cdot d^{-1}$) it is below the maximal safe level. Care should be taken however at this level of protein intake as other nutrient-rich foods may be displaced from the diet, leading to micronutrient deficiencies. Any such diet with an elevated protein intake, should also contain a wide range of whole grain cereals, fresh vegetables, and fruits, rich in micronutrients and potassium alkali salts needed to reduce the potential renal acid load and subsequent urinary calcium loss, that can occur due to the acidic nature of protein-rich diets. A more manageable and practical approach, which may still provide beneficial outcomes is a 25% protein energy diet, which would provide 118 g protein on an 8000 kJ/d diet at $1.5 \; g \cdot kg^{-1} \cdot d^{-1}$ for an 80 kg individual. This is clearly distinguishable from modern day popular *purported* "high protein diets," that are actually low in carbohydrate (25 to 90 g/d) and contain large amounts of total fat (50 to 60%), and saturated animal fat (30 to 50 g/d) (105, 106). These fad diets should not be mistakenly compared with the recommendations of this article, nor to the studies cited in this article which are neither high in fat nor low in carbohydrate (100-104), especially in light of recent minimum carbohydrate recommendations of 130 g/d (107).

Numerous studies have also shown improvements in blood lipid profiles on diets with increased protein intakes. O'Dea et al. (108) showed a marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines after temporary reversion to a traditional hunter-gather lifestyle, where energy derived from protein reached 54% (109). As the energy intake was relatively low (5040 kJ) the estimated daily protein intake in absolute terms was only 154 g/d (99) which equates to approximately $1.9 \; g \cdot kg^{-1} \cdot d^{-1}$ for an 80 kg individual. Other studies have shown that isoenergetic substitution of protein for carbohydrate can reduce total, LDL, and VLDL cholesterol and triacyclglycerides while increasing HDL cholesterol (5, 110).

Improvements in insulin sensitivity and maintenance of muscle mass have also been shown in obese women on hypo-energetic, elevated protein diets compared with hypo-energetic high carbohydrate diets (111). Recent epidemiological evidence

Table 3   Comparison of the Three Methods of Reporting Protein Consumption Levels, Absolute Amount in Grams/Day, Relative Amount As Percentage of Total Energy Consumed, and As the Daily Quantity Relative to Body Weight (g · kg⁻¹ · d⁻¹)

Daily energy intake (kJ/d)	% Energy as protein	Protein intake (g/d)	Body weight (kg)							
			40	50	60	70	80	90	100	110
			Protein intake (g · kg⁻¹ · d⁻¹)							
6000	15	53	1.3	1.1	0.9	0.8	0.7	0.6	0.5	0.5
	25	88	2.2	1.8	1.5	1.3	1.1	1.0	0.9	0.8
	35	124	3.1	2.5	2.1	1.8	1.5	1.4	1.2	1.1
8000	15	71	1.8	1.4	1.2	1.0	0.9	0.8	0.7	0.6
	25	118	2.9	2.4	2.0	1.7	1.5	1.3	1.2	1.1
	35	165	4.1	3.3	2.7	2.4	2.1	1.8	1.6	1.5
10,000	15	88	2.2	1.8	1.5	1.3	1.1	1.0	0.9	0.8
	25	147	3.7	2.9	2.5	2.1	1.8	1.6	1.5	1.3
	35	206	5.1	4.1	3.4	2.9	2.6	2.3	2.1	1.9
12,000	15	106	2.6	2.1	1.8	1.5	1.3	1.2	1.1	1.0
	25	176	4.4	3.5	2.9	2.5	2.2	2.0	1.8	1.6
	35	247	6.2	4.9	4.1	3.5	3.1	2.7	2.5	2.2
14,000	15	124	3.1	2.5	2.1	1.8	1.5	1.4	1.2	1.1
	25	206	5.1	4.1	3.4	2.9	2.6	2.3	2.1	1.9
	35	288	7.2	5.8	4.8	4.1	3.6	3.2	2.9	2.6

also shows an inverse correlation between protein intake and cardiovascular disease (CVD) in a cohort of 80,082 women (112). Dietary animal protein intake has been shown to be associated with lower plasma levels of the CVD risk factor, homocysteine (113), possibly through concomitant vitamin B-12 intake. Increased protein intake has also been associated with lower blood pressure in numerous population studies (114). A recent Japanese population study has also shown an inverse relationship between the level of protein consumption and stroke mortality (115).

The role of increased protein intake in the development and progression of renal dysfunction is a hotly debated issue. Numerous case studies show a clear increase in the rate of progression in renal dysfunction with increased protein ingestion. Certainly in cases of impaired renal function, reduced levels of protein intake can slow the progression to renal failure, however there is no link between increased protein intake (1.2 to 2.0 g · kg⁻¹ · d⁻¹) and development of renal insufficiency (17, 116), and renal clearance is still highly efficient at protein intakes of up to 3.0 g · kg⁻¹ · d⁻¹ (27). A recent clinical trial, for instance, has also shown that a diet with an elevated protein intake (26% of energy) has no adverse effects upon renal function in subjects with no pre-existing kidney disease (100).

Summary and Recommendations

Absorption rates of amino acids from the gut can vary from 1.4 g/h for raw egg white to 8 to 10 g/h for whey protein isolate. Slowly absorbed amino acids such as casein (~ 6 g/h) and repeated small doses of whey protein (2.9 g per 20 min, totaling ~ 7 g/h) promote leucine balance, a marker of protein balance, superior to that of a single dose of 30 g of whey protein or free amino acids which are both rapidly absorbed (8 to 10 g/h), and enhance amino acid oxidation. This gives us an initial understanding that although higher protein intakes are physiologically possible, and tolerable by the human body, they *may* not be functionally optimal in terms of building and preserving body protein. The general, although incorrect consensus among athletes and bodybuilders, is that rapid protein absorption corresponds to greater muscle building. Less is understood about protein and amino acid absorption from real whole foods, such as meat, chicken, fish, and vegetable-based proteins. Future studies should focus in this area as the majority of the population consume whole foods distinct from hydrolyzed proteins. It should be noted here, however, that the study of maximal rates of urea synthesis conducted by Rudman et al. (27) although comprehensive, were carried out on a limited sample size over 30 y ago, and future studies need to be carried out to safely verify these early findings. From the limited data available on amino acid absorption rates, and the physiological parameters of urea synthesis, the maximal safe protein intakes for humans have been estimated at ~ 285 g/d for an 80 kg male. It is not the intention of this article, however, to promote the consumption of large amounts of protein, but rather to prompt an investigation into what are the parameters of human amino acid kinetics. In the face of the rising tide of obesity in the Western world where energy consumption overrides energy expenditure, a more prudent and practical approach, which may still provide favorable outcomes, is a 25% protein energy diet, which would provide 118 g protein on an 8000 kJ/d diet at 1.5 g $\cdot$ kg^{-1} $\cdot$ d^{-1} for an 80 kg individual (Table 2).

In terms of people who participate in physical activity, retaining and building muscle is a primary goal. Diminished reserves of TCAI through restricted carbohydrate intake could potentially bring about an early onset of fatigue, decrease exercise performance, and promote muscle catabolism. As protein absorption of real foods is approximately 1 to 4 g/h, and fat is absorbed at approximately 14 to 18 g/h, the need for adequate glucose to prevent muscle gluconeogenesis and hence preserve lean muscle is important and further supports the need for a minimum carbohydrate intake, especially for active people. A carbohydrate intake of 120 to 150 g/d could be sufficient with active people consuming > 150 g/d from a large variety of cereals, whole grains, fresh fruit, and vegetables. Little data exists on the comprehensive metabolic effects of large amounts of dietary protein in the order of 300 to 400 g/d. Intakes of this magnitude would result in some degree of prolonged hyperaminoacidemia, hyperammonemia, hyperinsulinemia, and hyperglucagonemia, and some conversion to fat, but the metabolic and physiological consequences of such states are currently unknown. The upper limit of protein intake is widely debated, with many experts advocating levels up to 2.0 g $\cdot$ kg^{-1} $\cdot$ d^{-1} being quite safe (102, 117, 118) and that renal considerations are not an issue at this level in individuals with normal renal function. Based upon the current limited evidence available, the authors would speculate that 25% energy as protein is a safe and

viable level for the general public and athletes to both assist with weight control and maintain (or improve) lean body mass. However, the energy content of the diet and individual body weight must be considered. A maximum intake rate of 2.5 g · kg^{-1} · d^{-1} combined with the daily energy intake considerations shown in Table 2 would ensure absolute protein intakes well below potentially dangerous levels. For example, an 80 kg individual on a 25% protein energy intake would consume 176 g protein per day (2.2 g · kg^{-1} · d^{-1}) on a 12,000 kJ/d diet. A 60 kg individual would consume 118 g at 2.0 g · kg^{-1} · d^{-1} on a 8000 kJ/d diet and 147 g protein at 2.5 g · kg^{-1} · d^{-1} on a 10000 kJ/d diet. However, apart from pure quantitative issues, protein composition should be considered, as the importance of branched chain amino acids such as leucine may have important roles in metabolic regulation such as glucose homeostasis and muscle protein synthesis.

In conclusion, it is pertinent to include a quote from "The Second Workshop on the Assessment of Adequate Intake of Dietary Amino Acids" held in Honolulu, Hawaii, October 31 to November 1, 2002:

> The amounts of protein and, therefore, of amino acids consumed by humans vary over a wide range. When dietary nitrogen and essential amino acid intakes are above the requirement levels, healthy individuals appear to adapt well to highly variable dietary protein intakes, because frank signs or symptoms of amino acid excess are observed rarely, if at all, under usual dietary conditions. Thus, definition of tolerable ranges of amino acid intake in healthy people will require approaches that identify deviations from normal physiological and biochemical adaptive processes at the subclinical level. Further, the studies necessary to do so must conform to the strictest safety standards because of the ethical concerns of studying normal people (21).

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