Amino Acid Composition and Protein Contents of Selected Very Low Energy Reducing Diets†

Constantinos G. Zarkadas,*,‡,§ Errol B. Marliss,§ George C. Zarkadas,§ Ziran Yu,∥ Adolfo Minero-Amador,∥ James A. Rochemont,⊥ Shahrokh Khanizadeh,⊥,# and Rejeanne Gougeon§

Plant Breeding and Management Program, Plant Research Centre, Central Experimental Farm, Research Branch, Agriculture Canada, Ottawa, Ontario, Canada K1A 1C5, The McGill Nutrition and Food Science Centre, Royal Victoria Hospital, Montreal, Quebec, Canada H3A 1A1, Department of Biology, Nankai University, Tianjin, China, and Molecular and Biochemical Neuroendocrinology Laboratories, Clinical Research Institute of Montreal, Montreal, Quebec, Canada

The contents of total protein, amino acids including 5-hydroxylysine and 4-hydroxyproline, and estimated collagen of five very low energy reducing diets as therapeutic weight reduction treatment for the obese were determined as potentially useful indices for evaluating their protein quality. The amino acid composition and protein content of these diets varied with the amounts of plant or animal protein additives and ingredients used to formulate them. There were significant differences ($P < 0.001$) in their total essential amino acid contents, compared to the FAO/WHO reference pattern value of 33.9%, with the casein- and soybean-based diets ranging from 46.4 to 46.9% and the connective tissue-based diets varying from 12.7% to 18.8%. Calculated protein efficiency ratios ranged from 2.5 to 2.7 in the casein and soybean diets and from 1.0 to 1.4 in the connective tissue-based diets. Total collagen in connective tissue-based diets (51.3–65.5%) was determined from the amounts of 5-hydroxylysine found in their 96-h acid hydrolysates, and total connective tissue proteins (80.3–84.1%) were determined from the amounts of 4-hydroxyproline present. Very low energy diets when used without strict medical supervision can be life threatening.

INTRODUCTION

There has been a continuing interest in the development of low and very low energy diets for use as therapeutic weight reduction treatments for the obese [reviewed by Wadden et al. (1983, 1990), Fisler and Drenick (1987); Apfelbaum et al. (1970, 1987), and Bray (1989, 1990)]. Numerous commercial semistarvation diets are now available in packaged form, with a protein content ranging from 30 to 100 g of protein of either animal or plant origin and providing 400–600 kcal (1674–2510 kJ) of energy per day. Most of these diets include supplements of vitamins, minerals, and trace elements in amounts recommended for humans (Bolinger et al., 1966; Blackburn et al., 1975; Genuth et al., 1974; Sours et al., 1981; National Research Council, 1980).

Various studies have described a number of adverse effects caused by low or very low energy diets, including fatigue, weakness, postural hypotension, nausea, emotional disturbances, and, in more severe cases, cardiac arrhythmias associated with nitrogen depletion and/or potassium and other blood electrolyte imbalances (Wilson and Lamberts, 1979; Genuth, 1979; de Haven et al., 1980; Sours et al., 1981; VanItallie and Yang, 1984; Young et al., 1988). Although as many as 64 deaths associated with very low energy diets have been reported in the United States and another 3 deaths in Canada (Wadden et al., 1990; Health and Welfare, Canada, 1991), such reducing diets have continued to be used in the United States, Canada, and other countries under strict medical supervision. The principal advantages of using very low energy weight reduction regimens are as follows: First, they induce a negative energy balance, which results in a rapid and significant weight loss (Swendseid et al., 1965; Ravussin et al., 1985; VanItallie et al., 1990). Second, compared to total starvation, they tend to reduce the initial protein and mean muscle mass loss (Fisler et al., 1982, 1985; Fisler and Drenick, 1987). Third, after adaptation to such low energy and protein intakes, they tend to maintain the constancy of the body's nitrogen balance and protein turnover rates (Bistrian et al., 1977, 1981; Waterlow, 1986; Young et al., 1988, 1989; de Haven et al., 1980; Apfelbaum et al., 1970, 1987; Gougeon-Reyburn et al., 1989).

The metabolic response to fasting involves a gradual reduction in gluconeogenesis, increased fat mobilization, and ketogenesis which will meet the body's energy requirements, particularly in the brain, thus sparing the lean body tissue (Bristrian et al., 1981). This metabolic adaptation to a total fast can be enhanced by the introduction of dietary protein (Waterlow, 1986). It appears that this protein sparing effect is influenced by both the quality and quantity of the protein consumed (Marliss et al., 1978; Bistrian et al., 1981; Fisler et al., 1982). Typical formulations have included animal or plant proteins, such as milk and egg powders, meat, soybean, and other types of processed oilseed or vegetable protein products, some wheat, barley, and sesame proteins prepared by various separation and extraction procedures, and collagen or gelatin hydrolysates (i.e., beef hide extract). As a result, the protein quality and nutritive composition of such diets are highly variable, and this has become a subject of major interest to both the clinician and the food manufacturer as well as to the regulatory agencies con-

* Author to whom correspondence should be addressed [telephone (613) 995-3700 ext. 7635, 7510].
† Contribution No. 1410 from the Plant Research Centre.
‡ Agriculture Canada.
§ Royal Victoria Hospital.
∥ Nankai University.
⊥ Clinical Research Institute of Montreal.
Present address: St. Jean Research Station, Agriculture Canada, St. Jean, PQ, Canada.

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cerned with the development of standards for such protein products. An accurate assessment of the protein quality and nutritional adequacy of the commercially available low or very low energy diets is therefore essential.

MATERIALS AND METHODS

Materials. Types DC-6A (Lot 3280) and DC-5A (Lot 775) cation-exchange spherical resins, sized to 11.0 × 1.0 and 6.0 × 0.5 μm, respectively, were purchased from Dionex Chemical Co., Sunnyvale, CA. Alternative sources of spherical resins are Interaction Chemical Inc., Mountain View, CA, or Beckman Instruments Inc., Palo Alto, CA. The standard amino acid calibration mixture was purchased from Beckman Instruments, Inc., Palo Alto, CA. Other amino acids used as standards were obtained as follows: N^6-lysinolalanine [N^G-(DL-2-amino-2-carboxyethyl)-L-lysine] from Miles Analytical Laboratories, Inc., Elkhart, IN; the amino sugars D-glucosamine hydrochloride and D-galactosamine hydrochloride and 4-hydroxyproline from Calbiochem-Behring Corp., La Jolla, CA; L-tryptophan from Schwarz/Mann, Orangeburg, NY; norleucine from Pierce Chemical Co., Rockford, IL; and 3-nitro-L-tyrosine from Aldrich Chemical Co., Milwaukee, WI. Octanoic acid was obtained from Eastman Kodak Co., Rochester, NY, and phenol was a product of J. T. Baker Chemical Co., Phillipsburg, NJ. Reagents and buffers were made with high-purity laboratory water as described previously (Zarkadas et al., 1987). All other chemicals and reagents were of the highest purity commercially available and were used without further purification.

Experimental Procedures. *Reducing Diets.* The five very low energy reducing diets used for this study, which include Proti-15 cream-style chicken soup Nutri-15 chicken bouillon soup, Nutri-15 grapefruit beverage mix, Proti-Max chocolate flavored drink or pudding mix, and an edible collagen protein product (Poly-Pro), were obtained from Bariatrix International, Dorval, PQ, Canada. All of these diets are commercially available in packaged form containing 15 g of protein and 1 g or less of carbohydrates per serving (8 oz) and include supplements of vitamins, minerals, trace elements, DL-methionine, and/or L-tryptophan in amounts recommended for humans. Although the actual levels of various plant- or animal-derived protein ingredients being used in such processed diets were not disclosed by the manufacturer, one of these diets (Nutri-15) has been prepared from a mixture of partially hydrolyzed collagen and vegetable proteins. The other two protein products were made from either partially hydrolyzed gelatin (Nutri-15 cold drink mixes) or enzymatically hydrolyzed collagen (Poly-Pro). The remaining two were commercial preparations, one made of sodium and calcium caseinate (Proti-Max) and the other of isolated soybean protein (Proti-15).

Procedures for Amino Acid Analyses. Amino acid analyses were carried out on either a conventional (Beckman Model 120C) or an updated and fully automated amino acid analyzer (equivalent to Beckman Model 121MB) as described previously (Zarkadas et al., 1986, 1987). The automated instrument was interfaced with a Varian Vista 402 (Varian, Walnut Creek, CA) chromatographic data reduction system to enable both rapid quantitation of amino acids at the picomole range and accurate peak area analysis.

Complete amino acid analyses were carried out in each of three replicate samples of the very low energy diets. The dried samples or lyophilized powders (0.02 g) were hydrolyzed in Pyrex test tubes (18 × 150 mm) under vacuum (below 10 mmHg) with 5 mL of triple-glass-distilled constant-boiling HCl (6.0 M) at 110 °C in duplicate for 24, 48, 72, and 96 h, respectively, following the precautions described previously (Zarkadas et al., 1987, 1988a; Ozols, 1990). The data reported for serine and threonine represent the average of values extrapolated to zero time of hydrolysis. The values for valine, isoleucine, leucine, and phenylalanine are averages of data from 48, 72, and 96 h of hydrolysis. All others are reported as the average values from 24, 48, 72, and 96 h of hydrolysis.

The 4-hydroxyproline [Pro(4-OH)] content of the commercial diets was determined separately from a concentrated hydrolysate (equivalent to 0.1 mg of protein/analysis) as described previously (Berg, 1982; Zarkadas et al., 1986), and recoveries were calculated relative to alanine, leucine, and isoleucine present. Methionine and cysteine were determined separately (0.02-g samples) according to the performic acid procedure of Moore (1963). Norleucine was added in the hydrolysates as an internal standard, and the recoveries of cyst(e)ine as cysteic acid and methionine as the dioxide were calculated in proportion to the yields obtained by the performic acid treatment of standard solutions of these amino acids and relative to alanine, valine, leucine, and isoleucine present in the sample (Zarkadas et al., 1988a). Tryptophan in the very low energy diets (0.02 g) was determined separately after alkaline hydrolysis (Hugli and Moore, 1972) by an improved chromatographic procedure using 3-nitrotyrosine [Tyr(NO_2)] as an internal standard (Zarkadas et al., 1986).

Determination of the diastereoisomers of Lys(5-OH), lysinoalanine, and related compounds was carried out with concentrated hydrolysates (equivalent to 1–2 mg of protein) by the accelerated single-microcolumn (17.5 × 0.28 cm) system as described previously (Zarkadas et al., 1986; Stoscheck, 1990) so that peaks adequate for these components would be obtained.

Protein Determination. Precise quantitation of the protein concent in each of the reducing diet hydrolysates was carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a; Stoscheck, 1990). According to this method a mean residue weight (WE, in micrograms per nanomole) is calculated for the 18 standard amino acid residues plus Pro(4-OH) and Lys(5-OH) constituting the proteins in the low energy reducing diets using the expression

$$\mathrm{WE} = \sum_{i=1}^{20}(a_i b_i) \tag{1}$$

where a_i is the mole fraction of a specific amino acid i found in the analyzed aliquot and b_i is the molecular weight of amino acid residue i. A conversion factor CF (in micrograms per nanomole) was used for determining the protein mass in each hydrolysate sample analyzed in the absence of tryptophan and cyst(e)ine. Similarly, CF′, which is the apparent average residue molecular weight in micrograms per nanomole, was also used to calculate protein concentration in the absence of tryptophan, cyst(e)ine, proline, Pro(4-OH), and Lys(5-OH) and can be calculated as

$$\mathrm{CF}' = \sum_{i=1}^{15}(a_i b_i)/[1 - (a_{\mathrm{Trp}} + a_{\mathrm{Cys}} + a_{\mathrm{Pro}} + a_{\mathrm{Pro(4\text{-}OH)}} + a_{\mathrm{Lys(5\text{-}OH)}})] \tag{2}$$

These factors, WE, CF, and CF′, can be used in all subsequent quantitations of a given sample. The protein concentration P (in micrograms) of each hydrolysate was calculated by multiplying CF or CF′ by the total nanomoles (X_i) of amino acids found (Horstmann, 1979; Peterson, 1983) as follows:

$$P = \mathrm{CF}\sum_{i=1}^{18} X_i \tag{3}$$

Determination of Connective Tissue Proteins in the Diets. On the basis of known amino acid composition of purified collagen isoforms from the skin (Miller and Gay, 1982, 1987; Light, 1985, 1987), the content of collagen and collagen-like proteins can be determined from the amounts of Lys(5-OH) found (Zarkadas et al., 1988a). The following method was used to calculate collagen and connective tissue protein contents

$$P_i = \frac{1000}{c_i n'_i}\frac{\mathrm{WE}(P_j)}{M_{r(i)}} \tag{4a}$$

where WE(P_i) is the weight equivalent of a specific skin protein j, determined from eq 1 according to the method of Horstmann (1979) and Zarkadas et al. (1988a), c_i is the mean concentration in grams per kilogram of total protein-bound amino acid i found in the analyzed acid hydrolysate of the diets, n'_i is the number of residues of a unique amino acid residue per 1000 amino acid residues, and $M_{r(i)}$ is the anhydrous molecular weight of the unique amino acid i. Since the dry weight of skin is 40–50% collagen, and the types I and III collagens accounted, respectively, for 71.25 and 23.75% of the recovered collagen in the skin, while the less abundant types IV and V collagens accounted for the remaining 5.0% (Bailey and Sims, 1976; Light and Champion, 1984; Light, 1985, 1987; Light et al., 1985), a mean for the diastereoisomers of Lys(5-OH) content of n'_i = 10.0 residues per 1000 total amino acid residues in skin or bone collagen could be

Table I. Amino Acid Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of Three Collagen-Based Very Low Energy Reducing Diets

	reducing diets[a]							
	Poly-Pro edible collagen		Nutri-15 chicken bouillon mix		Nutri-15 grapefruit beverage mix		significant levels[a] among diets	
amino acid	mean ± SEM	CV	mean ± SEM	CV	mean ± SEM	CV	CV	$P > F$
aspartic acid	59.11 ± 0.73	2.13	62.82 ± 1.38[a]	3.79	59.90 ± 0.38	1.09	2.64	0.06
threonine	16.60 ± 0.29	3.02	17.60 ± 0.82	8.07	17.81 ± 0.04	0.45	5.02	0.26
serine	33.01 ± 0.72	3.79	32.49 ± 1.03	5.48	34.82 ± 0.55	2.75	4.11	0.17
glutamic acid	110.72 ± 0.71[b]	1.11	116.01 ± 6.18[a]	9.23	145.67 ± 2.44[a]	2.90	5.39	0.0014***
proline	142.18 ± 2.42[a]	2.95	106.57 ± 5.17[b]	8.40	104.03 ± 1.53[b]	2.55	5.02	0.0004***
glycine	205.77 ± 1.38	1.16	192.86 ± 8.13	7.30	202.27 ± 1.46	1.25	4.18	0.22
alanine	88.98 ± 0.37[a]	0.73	0.62 ± 2.35[a,b]	5.05	84.68 ± 0.57	1.17	2.88	0.01**
cysteine	3.47 ± 2.78	113.18	5.27 ± 2.89	95.08	1.90 ± 0.20	18.17	102.07	0.56
valine	26.44 ± 0.37	2.39	27.67 ± 4.67	10.45	26.11 ± 0.42	2.77	6.58	0.55
methionine	12.15 ± 3.33	47.49	16.91 ± 4.97	50.85	14.36 ± 1.20	24.09	43.55	0.67
isoleucine	11.59 ± 0.04	0.61	14.25 ± 1.97	23.95	13.72 ± 0.07	0.87	14.96	0.29
leucine	28.69 ± 0.22	1.30	32.04 ± 2.87	15.51	28.98 ± 0.13	0.76	9.63	0.35
tyrosine	7.01 ± 0.35	8.63	8.72 ± 1.96	38.91	6.85 ± 0.15	3.78	16.50	0.48
phenylalanine	19.66 ± 0.26[a,b]	2.29	21.92 ± 1.38[b]	10.91	18.23 ± 0.31[a]	2.99	7.23	0.05*
histidine	4.58 ± 0.22[b]	8.33	7.59 ± 1.64[a]	21.54	7.31 ± 0.14[a]	3.21	15.07	0.01**
lysine	37.42 ± 0.23	1.08	36.24 ± 2.16	10.30	37.11 ± 1.16	5.40	6.65	0.83
arginine	83.37 ± 0.97	2.02	80.44 ± 1.43	3.08	80.14 ± 0.30	0.65	2.16	0.12
tryptophan	0		2.79 ± 0.58	36.19	3.25 ± 0.17	8.97	24.61	0.49
4-hydroxyproline	100.02 ± 0.30	0.51	102.76 ± 1.33	2.24	104.75 ± 1.26	2.08	1.81	0.05*
5-hydroxylysine	10.35 ± 0.19[b]	3.12	8.13 ± 0.15	3.18	8.11 ± 0.04[c]	0.85	2.73	0.0001****
ammonia	12.24 ± 2.02	28.59	13.77 ± 5.62	70.75	13.26 ± 0.29	3.75	45.72	0.95
total protein								
g/kg of dry mass	947.72 ± 6.52[a]	1.19	782.03 ± 9.89[b]	2.19	766.07 ± 7.09[b]	1.60	1.66	0.0001***
WE, μg/nmol[e]	0.09142 ± 0.0002	0.42	0.09331 ± 0.0011	0.39	0.09266 ± 0.0001	0.34	1.27	0.21
CF, μg/nmol[e]	0.09142 ± 0.0002	0.42	0.09344 ± 0.0011	0.37	0.092806 ± 0.0002	0.35	1.26	0.18
CF′, μg/nmol[e]	0.11641 ± 0.0001[b]	0.10	0.11844 ± 0.0005[c]	0.11	0.113925 ± 0.0002	0.34[a]	0.44	0.0001****

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance, $P > F$ values: ****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; CV, coefficient of variation. [a–d] Means along a horizontal column with different superscripts are significantly different (Duncan, 1955). [e] Protein mass was determined according to the method of Horstmann (1979), and WE, CF, and CF′ constants were calculated according to the methods of Horstmann (1979) and Zarkadas et al. (1988a–c) using eqs 1–3.

computed from the relative distribution of collagen types and their respective Lys(5-OH) contents as described previously (Zarkadas et al., 1988a). The average residue weight (WE) for collagen is 91.1, and each of the diastereoisomers of Lys(5-OH) has an anhydrous M_r of 145.18. The following conventions, derived from eq 4a, can therefore be used for calculating collagen as grams per kilogram of total protein:

$$\text{amt of collagen } (P_c) = \text{amt of Lys(5-OH)} \times 63.3 \quad (4b)$$

An alternative method for calculating the amount of total connective tissue proteins in these diets (in grams per kilogram of total protein) could also be calculated from the known Pro-(4-OH) contents of collagen [$n'_i = 105.8$; see Zarkadas et al. (1988a)] and amorphous elastin ($n'_i = 22$) (Foster, 1982) and the anhydrous molecular weight of Pro(4-OH) ($M_{r(i)} = 113.12$). The following analytical convention, derived from eq 4a, can therefore be used for computing total connective tissue proteins (in grams per kilogram of total protein):

$$\text{amt of connective tissue } (P_{CT}) = \text{amt of Pro(4-OH)} \times 8.03 \quad (4c)$$

Statistical Analysis. Data processing and statistical analysis of the results were carried out by a FORTRAN computer program developed for this purpose. Analysis of variance conducted on the amino acid data for a completely randomized block design (factorial) was carried out according to the Statistical Analysis System (1982) general linear model procedure. Differences among sample means were also tested for significance with Duncan's multiple-range test (Duncan, 1955).

RESULTS AND DISCUSSION

Accurate and detailed amino acid determinations were carried out on five very low energy reducing diets to asceretain whether the amino acid profiles and/or collagen contents in such diets could be used as potentially useful indices for assessing their protein quality [Expert Work Group (FSIS), 1984; Pellett and Young, 1984, 1988; Young and Pellett, 1984; Lee et al., 1978; Nguyen et al., 1986; FAO/WHO, 1965; FAO/WHO/UNU, 1985; FAO/WHO, 1990). Three of these diets were semisynthetic hydrolysates of collagen (Poly-Pro and Nutri-15), one was casein-based (Proti-Max) and the other was a soybean-based diet (Proti-15). All of the diets included vitamins and mineral supplementation in amounts recommended for humans.

Results of the amino acid analyses of the five diets and the levels of statistical significance obtained from analysis of variance are presented in Tables I and II and are expressed as grams of anhydrous amino acid per kilogram of anhydrous fat- and ash-free tissue protein. The data represent the average values of three replicates ($N = 3$). Duplicate 24-, 48-, 72-, and 96-h hydrolysates were prepared, and each was analyzed in duplicate (48 determinations). The results show deviations of less than 2.5% from the average values obtained among the three replicates of each diet, but there were wide variations in the amino acid contents of the various diets evaluated. The method of reporting amino acid composition, as presented in Tables I and II, allows comparisons to be made between the results from this study, with those given in food-compositional tables, and the recommended FAO/WHO/UNU (1985) and FAO/WHO (1990) reference amino acid patterns for humans. This method has the added advantage that the percentage recovery of amino acids by weight can be found by simple summation (Tristram and Smith, 1963; Eastoe, 1967).

Another method for expressing amino acid content was based on grams of amino acid per 16 g of total nitrogen. This method was first introduced by Block and Weiss (1956) for rapid calculation of the amino acid content of diets in nutritional studies. For purposes of comparison

Table II. Amino Acid Composition and Protein Contents (Grams of Amino Acids per Kilogram of Total Protein) of a Casein-Based and a Soybean-Based Very Low Energy Reducing Diet

	reducing diets[a]					
	Proti-Max (casein-based) chocolate flavored drink or pudding mix		Proti-15 (soybean-based) cream-style chicken soup		significant level between diets	
amino acid	mean ● SEM	CV	mean ● SEM	CV	CV	$P > F$
aspartic acid	74.19 ± 1.21	2.82	84.28 ± 0.28	0.58	1.92	0.0012***
threonine	34.35 ± 1.33	6.73	37.33 ± 1.56	7.23	7.01	0.22
serine	48.96 ± 1.82	6.49	52.73 ± 0.99	3.25	4.99	0.14
glutamic acid	211.69 ± 5.17	4.23	221.66 ● 1.16	0.91	2.99	0.13
proline	100.58 ● 4.20	7.23	66.99 ± 4.69	12.13	9.20	0.006**
glycine	16.15 ± 0.65	7.03	24.09 ± 0.88	6.36	6.70	0.002***
alanine	26.18 ± 1.00	6.62	32.54 ± 0.71	3.82	5.14	0.007**
cysteine	7.33 ± 1.36	32.03	7.14 ● 2.08	50.49	42.05	0.94
valine	59.98 ± 1.83	2.13	59.84 ± 0.93	2.69	4.19	0.95
methionine	27.04 ± 0.29	1.87	22.52 ± 4.21	32.36	20.84	0.34
isoleucine	46.61 ± 0.57	2.13	48.93 ± 0.33	1.17	1.69	0.03*
leucine	83.13 ± 2.02	4.21	84.33 ± 1.93	3.97	4.09	0.69
tyrosine	51.83 ± 1.13	3.77	48.75 ± 0.98	3.49	3.64	0.11
phenylalanine	51.73 ± 1.69	5.67	48.63 ± 1.11	3.96	4.94	0.20
histidine	26.78 ± 0.77	5.02	27.62 ± 0.34	2.14	3.82	0.37
lysine	69.89 ± 1.92	4.75	71.20 ± 1.06	2.57	3.80	0.58
arginine	35.04 ± 1.04	5.14	48.49 ± 0.45	1.59	3.32	0.0003***
tryptophan	13.54 ● 0.11	1.38	11.86 ± 0.12	1.81	1.59	0.0005***
lysinoalanine	0.154 ● 0.008	9.70	0.165 ± 0.008	9.17	9.43	0.39
ammonia	12.31 ± 0.66	9.35	16.20 ± 2.31	24.74	20.69	0.18
total protein						
g/kg of dry mass	678.62 ± 5.69	1.45	654.67 ± 22.16	5.86	4.20	0.35
WE,[b] μg/nmol	0.124 699 ± 0.0006	0.91	0.114 586 ± 0.0003	0.46	0.72	0.87
CF,[b] μg/μmol	0.115 665 ± 0.0006	0.93	0.115 429 ± 0.0003	0.46	0.73	0.75
CF′,[c] μg/μmol	0.131 726 7 ± 0.0002	0.31	0.125 427 ± 0.0005	0.81	0.60	0.0006***

[a] Mean values ● standard error of measurements (SEM) for 3 replicates and 48 determinations. Significance: $P > F$ values: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; CV, coefficient of variation. [b] The weight equivalent (WE) and conversion factor (CF) were calculated according to the method of Horstmann (1979). [c] The conversion factor CF′ was also calculated according to the method of Horstmann (1979) using eq 2 but in the absence of tryptophan, cysteine, proline, and 4-hydroxyproline.

the data from this study have been calculated in this way and are presented in Table III.

A third method for reporting nitrogen content in diets was recommended by Heidelbaugh et al. (1975), and the results of the selected very low energy reducing diets are reported in Table III. The total nitrogen of the semisynthetic collagen-based diets averaged 19.07% (spread 18.22–20.09%), which is considerably higher than the 16.0% value frequently assumed for proteins and which serves as the basis for the factor of 6.25 used to convert total nitrogen to crude protein.

The data on the nitrogen content of the collagen-based reducing diets are in reasonably good agreement with those reported by Eastoe (1955, 1967) for the total nitrogen content of collagens and gelatin (18.0–18.6%) isolated from the extracellular matrices of a variety of connective tissues. The differences noted in the total nitrogen content between Poly-Pro and the two Nutri-15 diets may be attributed to the amino acid composition of the connective tissue proteins present. Significant differences ($P < 0.01$) in the total nitrogen values per kilogram of protein were found between the casein- and soybean-based diets, which varied from 15.58 to 16.33% N, respectively. To correct for this variation, new conversion factors based on the amino acid nitrogen content were calculated, which are characteristic for each of these diets and can be used in all subsequent quantitations for converting Kjeldahl nitrogen into crude protein content. Significant differences in protein conversion factors were also found among the various reducing diets evaluated, ranging from 6.12 in soybean-based diet (Proti-15) to 6.41 in casein-based diet (Proti-Max) and from 5.21 in edible collagen (Poly-Pro) to 5.25 and 5.27 in chicken bouillon and grapefruit beverage mixes (Nutri-15), respectively. These results give further support to the National Research Council's (1963) recommendation that the commonly used protein conversion factor of 6.25 is useful only for calculating the crude protein content of different foods.

Protein determinations in each acid hydrolysate were carried out according to the method of Horstmann (1979) as described previously (Zarkadas et al., 1988a,b), and the results are summarized in Tables I and II. This method of calculating the protein mass in diets or tissues is based upon a knowledge of the amino acid composition of the protein mixture and yields accurate estimates of the amount of protein present as determined by eqs 1 and 2. The mean residue weight (WE, micrograms per nanomole) and conversion factors CF and CF′ (micrograms per nanomole) given in Tables I and II can be used in all subsequent protein quantitations as described previously by Horstmann (1979) and Zarkadas et al. (1988a). The variation in the protein content among the three connective tissue-based reducing diets analyzed in this study was found to be highly significant ($P < 0.001$). Their protein content varied from 76.6–78.2% (dry weight basis; DWB) for the two Nutri-15 diets to 94.8% (DWB) for the Poly-Pro (edible collagen) diet, which reflects the amount of connective tissue proteins present.

There was little difference in the protein content between the soybean- and casein-based diets (Table II), but when these diets were compared with the collagen-based diets, the differences were highly significant ($P < 0.001$). Mean protein values (DWB) ranged from 67.8% in casein-based chocolate flavored mix (Proti-Max) to 65.4% in soybean-based cream-style chicken soup (Proti-15). The protein content of the soybean-based diet was midway between the protein content values reported previously by Zarkadas et al. (1988c) for soybean concentrate (59.5%) and isolate (76.2%).

Table III. Comparison of the Amino Acid Composition and Nitrogen Contents of Selected Very Low Energy Reducing Diets (Grams of Amino Acids per 16 g of Nitrogen)

	reducing diets/protein source[a]						
	animal origin				plant origin		
		Nutri-15 (connective tissue-based)		Proti-Max (casein-based)	Proti-15 (soybean-based)	significance level among reducing diets	
amino acid	Poly-Pro edible collagen	chicken bouillon mix	grapefruit beverage mix	chocolate flavored drink	cream-style chicken soup	CV	$P > F$
aspartic acid	4.930 ± 0.086[d]	5.034 ± 0.025[d]	5.307 ± 0.228[d]	7.456 ± 0.275[c]	8.262 ± 0.111[b]	4.81	0.0001****
threonine	1.385 ± 0.032[c]	1.497 ± 0.006[c]	1.485 ± 0.071[c]	3.631 ± 0.172[b]	3.662 ± 0.187[b]	8.83	0.0001****
serine	2.754 ± 0.081[c]	2.927 ± 0.048[c]	2.751 ± 0.130[c]	4.999 ± 0.385[b]	5.172 ± 0.149[b]	9.23	0.0001****
glutamic acid	9.233 ± 0.035[d]	12.243 ± 0.222[c]	9.069 ± 0.182[d]	21.296 ± 0.899[b]	21.730 ± 0.342[b]	5.29	0.0001****
proline	11.855 ± 0.191[e]	8.743 ± 0.132[d]	8.992 ± 0.314[c,d]	10.232 ± 0.801[c]	6.578 ± 0.468[b]	8.39	0.0001****
glycine	17.159 ± 0.114	16.998 ± 0.104[b]	16.266 ± 0.626	1.664 ± 0.069[c]	2.363 ± 0.076[c]	4.64	0.0001****
alanine	7.420 ± 0.033[b]	7.117 ± 0.044[c]	6.797 ± 0.626[d]	2.680 ± 0.089[f]	3.192 ± 0.101[e]	2.07	0.0001****
cysteine	0.291 ± 0.232	0.160 ± 0.0168	0.457 ± 0.257	0.882 ± 0.263	0.704 ± 0.199	69.84	0.187[ns]
valine	2.20 ± 0.024[c]	2.195 ± 0.034[c]	2.342 ± 0.194[c]	6.165 ± 0.176[b]	5.868 ± 0.147[b]	6.26	0.0001****
methionine	1.014 ± 0.027[d]	1.207 ± 0.168[c,d]	1.443 ± 0.456[c,d]	2.825 ± 0.064[b]	2.199 ± 0.386[b,c]	30.47	0.0001****
isoleucine	0.996 ± 0.006[c]	1.153 ± 0.007[c]	1.211 ± 0.195[c]	4.887 ± 0.122[b]	4.796 ± 0.072[b]	7.19	0.0094**
leucine	2.392 ± 0.004[c]	2.435 ± 0.014[c]	2.715 ± 0.306[c]	8.600 ± 0.232[b]	8.269 ± 0.271[b]	7.46	0.0001****
tyrosine	0.584 ± 0.025[d]	0.576 ± 0.012[d]	0.743 ± 0.184[d]	5.370 ± 0.133[b]	4.778 ± 0.113[c]	8.19	0.0001****
phenylalanine	1.639 ± 0.023[d]	1.532 ± 0.025[d]	1.856 ± 0.162[d]	5.292 ± 0.142[b]	4.768 ± 0.126[c]	6.46	0.0001****
histidine	0.382 ± 0.016[d]	0.614 ± 0.011[c]	0.644 ± 0.094[c]	2.787 ± 0.098[b]	2.707 ± 0.061[b]	8.19	0.0001****
lysine	3.121 ± 0.044	3.118 ± 0.093	3.069 ± 0.260	7.226 ± 0.219[b]	6.978 ± 0.127[b]	6.22	0.0001****
arginine	6.954 ± 0.131[b]	6.735 ± 0.035[b]	6.787 ± 0.135[b]	3.624 ± 0.119[d]	4.753 ± 0.055[c]	3.12	0.0001****
tryptophan	0	0.273 ± 0.014[d]	0.237 ± 0.053[d]	1.415 ± 0.027[b]	1.162 ± 0.023[c]	30.48	0.0094**
4-hydroxyproline	8.341 ± 0.078	8.804 ± 0.102	9.523 ± 0.994	0	0	11.27	0.403[ns]
5-hydoxylysine	0.863 ± 0.022[b]	0.682 ± 0.004[c]	0.687 ± 0.028	0	0	4.76	0.0012**
lysinoalanine	0	0	0	0.016 ± 0.001	0.016 ± 0.001	11.13	0.874[ns]
ammonia	1.018 ± 0.160	1.114 ± 0.023	1.137 ± 0.429	1.206 ± 0.020	1.574 ± 0.213	32.40	0.499[ns]
total AA N[g]							
g of AA N/kg of protein	191.890 ± 1.562[b]	190.382 ± 0.269[b]	189.806 ± 5.68[b]	155.869 ± 0.519[c]	163.307 ± 1.742[c]	2.68	0.0001****
g of AA N/kg of dry mass	181.838 ± 0.411[b]	145.613 ± 1.516[c]	148.321 ± 6.10[c]	103.329 ± 1.856[d]	106.278 ± 4.477[d]	4.49	0.0001****
g of AA/16 g of N	83.392 ± 0.676[d]	84.042 ± 0.119[d]	84.444 ± 2.469[d]	102.713 ± 0.392[b]	98.013 ± 1.068[c]	2.39	0.0001****

[a] Mean values and standard error of measurements (SEM) for 3 replicates ($N = 3$) and 48 determinations. Significance, $P > F$ values: ****, $P < 0.0001$; **, $P < 0.01$; CV, coefficient of variation. [b-f] Means along a horizontal column with different superscripts are significantly different (Duncan, 1955). [g] Total amino acid nitrogen was determined according to the methods of Heidelbaugh et al. (1975), Horstmann (1979), and Zarkadas et al. (1988a–c).

The results summarized in Tables I and II show that the best estimate of the protein content in each of these low energy reducing diets was made by the summation of the weights of the amino acid residues present and that this method yields accurate estimates of the absolute amount of protein in the diets evaluated. Similarly, the data in Table III showed the most sensitive and least variable method for determining the total protein nitrogen contents of the selected plant- or animal-based diets to be a summation of the weights of the amino acid nitrogen present in each diet, as determined by detailed amino acid analysis (Heidelbaugh et al., 1975).

The amino acid profiles of the three collagen-based reducing diets, i.e., chicken bouillon soup and grapefruit beverage mix (Nutri-15) and edible collagen (Poly-Pro; Tables I and III), appeared to be very similar in composition. Glycine was the most abundant amino acid, accounting for almost 17.7–20.3% of all residues. Proline and 4-hydroxyproline taken together accounted for a further 20.0–23.6%, with glutamic acid at 10.0% and aspartic acid representing approximately 6.0% of all residues, giving a frequency of carboxy groups of about 16.0–17.0%. Since there are some 40–44 amide groups per thousand residues in collagen (Eastoe, 1967), the frequency of the free carboxyl groups is approximately 12–13%. The frequency of total basic amino acids, which include lysine, histidine, arginine, and 5-hydroxylysine, is approximately 13.3–15.6% and exceeds that of the free carboxyl groups. This indicates that the three collagen-based reducing diets contained predominantly basic proteins, which agrees with the finding of Eastoe (1967) that native collagen has an isoionic point above pH 9.0. The high content of 4-hydroxyproline, together with the small amounts of 5-hydroxylysine (0.79–1.01%) and tyrosine (0.64–0.66%), brings the total content of amino acid residues with hydroxyl groups to nearly 11.4%, which is relatively high compared with the majority of proteins. The extremely wide range of frequency of occurrence of amino acids from glycine (1 in 4) to tyrosine is highly characteristic of the amino acid composition of collagen from various mammals (Eastoe, 1955, 1963). The small amounts of cyst(e)ine and tryptophan present in these diets indicate that these two amino acids are possible constituents of other extracellular matrix protein components, i.e., fibronectin, laminin, etc., present in these diets rather than true constituent amino acids of collagen present.

The casein-based Proti-Max diet, which is often marketed as a pudding and shake product, has an overall amino acid profile which distinguished it from the collagen- and soybean-based diets investigated. Proti-Max is rich in glutamic acid (20.5%), proline (9.5%), leucine (8.3%), valine (5.7%), and the basic amino acids, which account for a further 12.7% of all residues (Tables II and III). Aspartic acid accounts for 7.2% of the amini acid residues. Although the data reported in Tables II and III are in reasonable agreement with those reported by FAO/WHO (1965) for cow's milk and by Zarkadas et al. (1988c) for milk solid nonfat powder, some differences were noted. The threonine and cystine contents in Proti-Max were lower than the corresponding cow's milk values quoted in Table V from FAO/WHO (1965). The lysine content of Proti-Max did not approach that of cow's milk. There is a lower content of the long-chain amino acids valine, isoleucine, and leucine in this diet compared to the reference pattern, and methionine was present in sub-

Table IV. Protein Quality Evaluation of Selected Very Low Energy Reducing Diets Based on Their Detailed Amino Acid Composition Data

	reducing diets/protein source[a]						
	animal origin				plant origin		
	Poly-Pro edible collagen	Nutri-15 (connective tissue-based) chicken bouillon mix	Nutri-15 (connective tissue-based) grapefruit beverage mix	Proti-Max (casein-based) chocolate flavored drink or pudding	Proti-15 (soybean-based) cream-style chicken soup	significance level among reducing diets CV	significance level among reducing diets $P > F$
(i) essential amino acids (EAA)							
total EAA,[e] mg/g of nitrogen	1278.5 ± 30.13[c]	1305.1 ± 4.39[c]	1396.46 ± 125.9[c]	3081.8 ± 65.92[b]	2996.2 ± 37.4[b]	5.78	0.0001****
EAA index[f]	20.24 ± 0.54[d]	30.88 ± 0.35[c]	32.63 ± 3.44[c]	85.18 ± 1.85[b]	82.52 ± 0.39[b]	8.47	0.01**
chemical score[f]	0	16.60 ± 1.19	22.71 ± 1.34[c]	32.63 ± 5.17[b]	31.67 ± 9.30[b]	56.41	0.007**
EAA_7, % of total protein[g]	14.04 ± 0.02[c]	14.20 ± 0.16[c]	14.97 ± 0.93[c]	34.57 ± 0.84[b]	35.03 ± 0.61[b]	4.84	0.0001****
EAA_{10}, % of total protein[g]	22.84 ± 0.071[c]	23.26 ± 0.13[c]	24.05 ± 0.86[c]	42.10 ± 1.03[b]	43.82 ± 0.59[b]	3.68	0.0001****
(ii) protein efficiency ratio (PER)[g] predicted by							
eq 4 (PER_7)	1.026 ± 0.002[c]	1.038 ± 0.013[c]	1.100 ± 0.055[c]	2.685 ± 0.068[b]	2.722 ± 0.049[b]	5.15	0.0001****
eq 5 (PER_{10})	1.289 ± 0.005[c]	1.316 ± 0.008[c]	1.366 ± 0.055[c]	2.507 ± 0.065[b]	2.616 ± 0.038[b]	3.99	0.0001****
eq 10 ($PER_{collagen}$)	1.314 ± 0.005	1.227 ± 0.008	1.263 ± 0.005				
(iii) connective tissue proteins							
collagen content,[h] % total protein	655.27 ± 11.81[b]	513.30 ± 2.53[c]	514.82 ± 9.43[c]			4.75	0.0012**
connective tissue content,[i] % total protein	803.16 ± 2.37	841.14 ± 10.08	825.1 ± 10.68			11.27	0.4033[ns]

[a] Mean values ± standard error of measurements (SEM) for 3 replicates and 48 determinations. Significance, $P > F$ values: ****, $P < 0.0001$; **, $P < 0.01$; CV, coefficient of variation. [b-d] Means along a horizontal column with different superscripts are significantly different (Duncan, 1955). [e] Computed from reference protein standards (FAO/WHO, 1965). [f] Calculated according to the methods of Block and Mitchell (1946) and Oser (1961). [g] Calculated according to the method of Lee et al. (1978) and Pellett and Young (1984). EAA_7, threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine. EAA_{10}, EAA_7 plus histidine, arginine and tryptophan. PER_7 values were calculated according to the method of Lee et al. (1978) from eq 5 [$PER = 0.08084(EAA_7) - 0.1094$], eq 6 [$PER = 0.06320(EAA_{10}) - 0.1539$], and eq 7 [$PER_{COL} = 0.02290(COL) + 3.1528$], where COL is the percentage of total protein. [h] The total collagen content was calculated from the amount of Lys (5-OH) using eq 4b. [i] The total connective tissue content of the reducing diets was calculated from the amounts of Pro (4-OH) present with eq 4c.

stantially higher quantity in Proti-Max than in cow's milk. It is not known, however, whether free methionine was included with this diet as supplement.

The soybean-based diet (Nutri-15) contained significant amounts of all amino acids commonly found in proteins with the exception of methionine and cyst(e)ine (Tables II and III). The acidic amino acids were present in high quantities and when taken together accounted for almost 30.5% of all residues in the reducing diet. The basic amino acids accounted for a further 14.7%. The present mean values for aspartic acid and arginine for the Proti-15 diet were lower than the values found in texture soybean flour Promate, concentrate, and isolate by Zarkadas et al. (1988c). The aromatic amino acids tyrosine and phenylalanine were present in approximately the same amounts. While these results are in reasonable agreement with those listed by Zarkadas et al. (1988c) for texture soybean flour Promate, the differences noted in amino acid content of this soybean-based diet indicated that, in addition to soybean proteins, formulations must have included a number of other plant or animal protein additives and ingredients to enhance the texture and reduce the cost of this diet.

The presence of small amounts of the unique amino acid lysinoalanine in the acid hydrolysates of both the casein- and soybean-based diets (Proti-Max and Proti-15), ranging from 0.154 to 0.168 g/kg of total protein (Tables II and III), is of interest since this cross-linked amino acid could have an adverse effect on the biological value of proteins (Maga, 1984). The present lysinoalanine values in casein- and soybean-based reducing diets (Tables II and III) are in accord with those reported by others for soybean and milk proteins (Friedman, 1977; Schwass and Finley, 1984; Maga, 1984). For example, alkaline-treated soybean meal and protein isolates have been reported to contain 0.57 and 0.80 g of lysinoalanine/16 g of nitrogen, respectively (de Groot and Slump, 1969), compared to alkaline-treated casein, which contained 1.15 g of lysinoalanine/g of nitrogen (de Rham et al., 1977). Although alkaline treatment of casein and soybean proteins brings about desirable changes in flavor, texture, and solubility, it also produced undesirable changes in the constituent amino acids, which include cross-linking formation, browning reaction products, and racemization of amino acids (Friedman, 1991; Friedman et al., 1991). The formation of lysinoalanine in foods has been primarily associated with the alkaline treatment or heat processing of proteins (Sternberg et al., 1975) and has been reported to be nephrotoxic to rats (Woodard and Short, 1974; 1977; Masters and Friedman, 1979). It has also been shown to inactivate metalloenzymes such as carboxypeptidases A and B and yeast alcohol dehydrogenase by removing the zinc ion from their active site at millimolar concentration (Hayashi, 1982). Toxicity of lysinoalanine to humans, however, has not been reported.

The essential amino acid (EAA) profiles of the three very low energy collagen-based diets ranged from 1279 to 1396 mg of EAA/g of dietary nitrogen (Table IV). The data indicated that these diets contained significantly lower amounts of all EAA required for human nutrition than either whole egg (3215 mg of EAA/g of nitrogen) or cow's milk protein (3200 mg of EAA/g of nitrogen) (FAO/WHO, 1965). Similar results were obtained from the EAA indices and protein scores (Table IV) of these connective tissue-based diets, calculated from their amino acid composition (Tables I–III) according to the methods of Block and Mitchell (1946) and Oser (1951). The low EAA indices (i.e., Poly-Pro, 20.2; Nutri-15, 32.6) and protein score values (16.6–22.7) of these three connective tissue-based diets reflect their deficits in tryptophan, histidine, and the sulfur-containing and aromatic amino acids, as shown in Table I. These results are in close agreement with earlier findings by Nguyen et al. (1986) on porcine skin, which indicated that skin connective tissue proteins

Table V. Comparison of the Essential Amino Acid (EAA) Composition of Five Selected Very Low Energy Reducing Diets and High-Quality Animal Proteins with the Suggested EAA Pattern of Requirements for Humans

			essential amino acid composition							
			reducing diets							
	EAA pattern of requirement[a]			Nutri-15						
							animal products			
EAA	preschool child (2–5 years)	adult	Poly-Pro edible collagen	chicken bouillon mix	grapefruit beverage mix	Proti-max (casein-based)	Proti-15 (soybean-based)	egg[b]	cow's milk[b]	beef[c]
	Milligrams of Amino Acid per Gram of Total Protein									
histidine	19	16	4.6	7.6	7.3	27	28	22	27	34
isoleucine	28	13	11.6	14.3	13.7	47	49	54	47	48
leucine	66	19	28.7	32.0	29.0	83	84	86	95	81
lysine	58	16	37.4	36.2	37.1	70	71	70	78	89
methionine and cystine	25	17	13.9	19.5	15.3	31	26	57	33	40
phenylalanine and tyrosine	63	19	26.7	30.6	25.1	103	97	93	102	80
threonine	34	9	16.6	17.6	17.8	34	37	47	44	46
tryptophan	11	5	0	2.8	3.3	14	12	17	14	12
valine	35	13	26.4	7.7	26.1	60	60	66	64	50
total										
including His	339	127	161.0	188.3	174.6	469	464	512	504	479
minus His	320	111	156.4	180.7	167.3	442	436	490	477	445
			Percent Protein Digestibility in Man							
			90	90	90	95	86	95	97	98
	Percent Amino Acid Score Adjusted for Digestibility (Percentage of Adequacy)[b]									
for preschool child (2–5 years)			23	24	28.5	95	93	119	119	94
adult			27.5	44	44.0	100	100			

[a] Data from FAO/WHO/UNU (1985), FAO/WHO (1990), and Bodwell (1987). [b] Calculation of protein rating was carried out by comparison of the amino acid composition of the selected very low energy reducing diets with that of the reference pattern established by FAO/WHO/UNU (1985) for preschool child (2–5 years) and adult. [c] Data taken from Bodwell (1987).

were limiting with respect to tryptophan, cyst(e)ine, tyrosine, and isoleucine. The present data (Table IV) also indicate that only the casein- and soybean-based protein diets evaluated in this study (Proti-Max and Proti-15) contained all of the EAA required for human nutrition, with mean values for total EAA ranging from 2996 to 3081 mg/g of nitrogen. Although lower than those of cow's milk and whole egg proteins, the values were considerably higher than the levels reported for the connective tissue-based diets in Table IV.

Despite the obvious advantages of simplicity and widespread use of these chemical scoring methods, which are based primarily upon a knowledge of the levels and distribution of the constituent essential amino acids of a protein or protein mixture, such values fail to take into account differences in the quality and digestibility of the various proteins present or the availability of individual amino acids. A more accurate assessment of the protein quality of foods, especially of protein mixtures containing connective tissue proteins, was recommended by the USDA [Expert Work Group (FSIS), 1984], Alsmeyer et al. (1974), Happich et al. (1975), Lee et al. (1978), and Pellett and Young (1984, 1988). It involves both the determination of the complete amino acid composition and total collagen content. These and other data were used by FAO/WHO/UNU (1985) to develop reference amino acid patterns for four different age groups (infants, 2–5-year-old children, 10–12-year-old children, and adults). They recommended that, in conjunction with in vivo protein digestibility data, the most appropriate approach would be to use amino acid values for the 2–5-year-old child as the reference pattern (Table V) in the evaluation of mixed diets for all persons except infants. The nine essential amino acids used include histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine (FAO/WHO/UNU, 1985). Since cystine and tyrosine can partially replace methionine and phenylalanine, respectively, the two sulfur-containing (methionine plus cystine) and two aromatic amino acids (phenylalanine and tyrosine) are usually considered together.

The results in Table IV indicate that the five diets evaluated had significant differences in their total EAA_7 and EAA_{10} contents, with the highest amounts being present in the casein- and soybean-based diets (Proti-Max and Proti-15) and the lowest amounts in the connective tissue-based diets (Poly-Pro and Nutri-15). Mean values for total EAA_7 in casein- and soybean-based diets ranged from 34.5 to 35% and for total EAA_{10} from 42 to 43.8%. Moreover, in using the prediction eqs 5 (EAA_7) and 6 (EAA_{10}) of Lee et al. and Pellett and Young (1984), the results summarized in Table IV show that the calculated protein efficiency ratios (PERs) for casein- and soybean-based diets ranged from 2.5 to 2.7, respectively. The high PER values observed for these diets are indicative of the complementation of the plant and animal proteins present. By contrast, the mean values for total EAA_7 and EAA_{10} were found to be very low in the connective tissue-based diets and ranged from 14.0 to 14.9% and from 22.8 to 24%, respectively. Similarly, the calculated mean PER values for the connective tissue-based diets (Poly-Pro and Nutri-15) were also low and ranged from 1.0–1.25 for the enzymatically hydrolyzed collagen (Poly-Pro) to 1.1 and 1.3 in the partially hydrolyzed gelatin (Nutri-15). These results show that the calculated mean PER values for connective tissue-based diets, which for animal proteins, i.e., whole egg, milk, etc., averages 3.2, also varied with the amounts of collagen present as a percentage of dietary protein.

The essential amino acid profiles and protein ratings of the five very low energy reducing diets investigated are compared with those of the reference pattern (FAO/WHO/UNU, 1985) for a 2–5-year-old child and with three high-quality animal proteins such as hen's whole egg, cow's milk, and bovine skeletal muscle tissue, and the results

are shown in Table V. These diets varied 3–4-fold in amino acid scores. The casein- and soybean-based diets have true protein digestibilities of 95 and 86%, respectively (FAO/WHO, 1990). Mean values for corrected amino acid scores ranged from 95% in casein-based diets to 93% in soybean-based diets. These two diets would provide adequate amounts of all of the essential amino acids ranging from 46.4 to 46.9%, which is considerably higher than the 33.9% reference pattern value given by FAO/WHO (1990). These results correspond closely with the mean essential amino acid values calculated according to the methods of Lee et al. (1978) and Pellett and Young (1984).

The calculated amino acid scores for the connective tissue-based diets are low, ranging from 23 in Poly-Pro to 24.0 and 28.5% in chicken bouillon and grapefruit beverage mixes, respectively. These low values probably result from deficiencies in most essential amino acids, especially tryptophan and histidine. Laser-Reutersward et al. (1982, 1985a,b) have shown that over 90.0% of the protein present in pig skin or bovine tendon was digestable by the rat, regardless of the heat treatment during processing or the age of the animal from which these tissues were taken. Since this method is based on human amino acid requirements, the protein digestibility-corrected amino acid scores presented in Table V appear to be a more appropriate procedure for evaluating protein quality of diets than animal assays.

In this study an attempt was also made to relate the protein quality of the three connective tissue-based diets to the amounts of the unusual protein-bound amino acids 5-hydroxylysine and 4-hydroxyproline using the single-column chromatographic method developed in this laboratory (Zarkadas et al., 1986). In this chemical approach, collagen is determined directly from the amounts of 5-hydroxylysine found in the acid hydrolysates of these diets using eq 4b. Total connective tissue proteins, which include collagen, elastin, etc., are determined from the amounts of 4-hydroxyproline present multiplied by 8.03 (eq 4c). The results, summarized in Table IV, show that in the three diets evaluated by this method there were significant differences in their total collagen contents ($P < 0.001$), with the highest amounts of collagen found in the Poly-Pro diet, which contained mostly enzymatically hydrolyzed collagen, compared to the two Nutri-15 diets, which consisted of partially hydrlyzed gelatin.

Mean values for total collagen in the Poly-Pro diet averaged 65.5% of the total protein in the diet compared to 51.3–51.4% collagen found in the chicken bouillon and cold drink mixes (Nutri-15). The differences noted in collagen, glycine, alanine, and arginine contents (Tables I and IV) suggest that, in addition to collagen, the Nutri-15 diets must contain various other proteins that are relatively higher in certain amino acids, which have little or no 5-hydroxyllysine. These results are in good agreement with those reported for pig skin collagen (63.3%) by Nguyen et al. (1986). When the 4-hydroxyproline contents of these diets were used, the values for total connective tissue proteins were higher and averaged 80.3 and 82.5–84.1% for Poly-Pro and Nutri-15, respectively (Table IV). Although the data reported for the connective tissue contents of these diets in Table IV (80.3–84.1%) are in reasonable agreement with the values 75.9–78.9% reported by Laser-Reuterward et al. (1982, 1985a) and Nguyen et al. (1986) for pig skin, they differ considerably from those reported for bovine hide collagen (90–95%) by Bowes et al. (1955, 1957). The higher collagen value of 65.5% for enzymatically hydrolyzed collagen (Poly-Pro), compared to 51.4% collagen for partially hydrolyzed gelatin (Nutri-15), calculated from the data of Light (1985, 1987), may be attributed to the purity of gelatin used in these diets, since the calculated connective tissue values in all cases appear to be very similar. The accuracy of such calculations, however, will depend on the purity of the collagen on which their 5-hydroxylysine and 4-hydroxyproline contents are based. The ratio of type I to type III collagen in skin is normally 4:1 (Bailey and Sims, 1976). The possibility remains, of course, that there exist minor differences in the extent of lysine hydroxylation between the collagens of pig and calf skins or tendons used in the preparation of these diets and that some of the 5-hydroxylysine could be converted to lysinoalanine during alkaline treatment.

The data presented in this paper show the variations that exist in the amino acid composition and protein content among selected very low energy diets that are being marketed today as therapeutic weight reduction treatments for the obese. Each of these diets has a characteristic amino acid profile, reflecting the amounts of plant or animal protein additives and ingredients used to formulate it. From these results it may be concluded that a potentially useful method for evaluating the protein quality of different diets might be based on a knowledge of their amino acid composition, and/or connective tissue protein contents, as recommended by FAO/WOHO/UNU (1985), FAO/WHO (1990), Pellett and Young (1984, 1988), Bodwell (1987), Zarkadas et al. (1990, 1992), and Zarkadas (1992).

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