New Protein Foods in Human Health:

NUTRITION, PREVENTION, AND THERAPY

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Chapter 10

SOY PROTEINS AS AMINO ACID AND PROTEIN SOURCES FOR PRESCHOOL-AGE CHILDREN

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I. INTRODUCTION

New sources of protein foods are needed for optimal nutrition and health of populations with diets that are lacking or marginal in terms of total nitrogen and essential amino acid contents. This need grows with the increase in cost of traditional protein sources and with the changes in eating habits induced by urbanization, migrations, and cultural changes. Vegetables and soy, in particular, are economic sources of protein that can be used to increase the supply of dietary protein and/or improve the balance of essential amino acids through combinations that complement the limiting amino acids of the various protein sources in the diet.[1,2]

After the infant, the child of preschool age is the most sensitive in terms of essential amino acid requirements. The primary needs are for tissue maintenance and body functions. Growth requires a relatively small, but highly important proportion of the total amino acid needs. Where food resources are limited or its distribution is unequal, protein and amino acid restrictions during this critical age can result in a reduction of body size, often accompanied by functional limitations.

II. ESSENTIAL AMINO ACID REQUIREMENTS AND RECOMMENDATIONS

Prior to 1981, the Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committees on protein requirements did not have available adequate experimental data of the essential amino acid requirements of preschool-age children. Beyond early infancy, the only existing information was from the studies of Nakagawa and co-workers[3,4] with children 10 to 12 years old. The 1971 committee[5] estimated the requirements of preschool children by interpolation between those of infants and the 10- to 12-year olds. It also recognized that the protein value of a food could be estimated from its essential amino acid content in relation to an amino acid pattern of reference, which was based on the estimated requirements of preschool children. In 1975, it was further recommended that this protein value should be corrected by the digestibility of the protein source in relation to that of milk and egg as reference proteins.[6]

The lack of experimental evidence on the amino acid requirements of preschool children led to a series of studies conducted by Torun et al.[7] and Pineda and co-workers[8] at the Institute of Nutrition of Central America and Panama (INCAP). They used semielemental diets based on a core level of cow milk and synthetic amino acids. The amounts of each specific essential amino acid under study were gradually increased or decreased. The children's requirements were established from serial analyses of free amino acids in plasma, nitrogen balance, and urinary urea/creatinine ratio. Further studies using cow milk and isolated soy proteins to determine protein requirements of similar children[9,10] confirmed the findings and suggested that the amounts of essential amino acids derived from the studies with semielemental diets could in fact be considered as "recommended" or "safe levels" of intake.[7] Those amounts are shown in Table 1.

III. SUGGESTED PATTERN OF AMINO ACID REQUIREMENTS

The 1981 FAO/WHO/United Nations University (UNU) Expert Committee on energy and protein requirements[11] accepted the recommendations proposed by Torun and co-workers.[7] The daily safe level of protein intake for children 2 to 5 years old was taken as 1.10 g reference (milk) protein per kilogram. Dividing the recommended levels of essential amino acids by 1.10 gave origin to the amino acid pattern of requirements suggested for preschool children (Table 2). The "provisional pattern" suggested in 1973 and the amino acid composition of soy protein are shown for comparison.

TABLE 1
Recommended Essential Amino Acid Intakes for Preschool Children

Amino Acid	Recommended intake (mg/kg/d)
Histidine[a]	
Isoleucine	31
Leucine	73
Lysine	64
Methionine + cystine	27
Phenylalanine + tyrosine	69
Threonine	37
Tryptophan	12.5
Valine	38

Note: Must be corrected for digestibility of protein source relative to milk.

[a] Requirement for histidine not measured.

From Torun, B., Pineda, O., Viteri, F. E., and Arroyave, G., in *Protein Quality in Humans: Assessment and In Vitro Estimation,* Bodwell, C. E., Adkins, J. S., and Hopkins, D. T., Eds., AVI Publishing, Westport, CT, 1981, 374. With permission.

TABLE 2
Current and Previously Suggested Patterns of Essential Amino Acid Composition of Proteins (Milligrams Amino Acid Per Gram Protein)

Amino acid	Current pattern (1985)[a]	Provisional pattern (1973)	Isolated soy protein[b]
Histidine	(19)[c]	—	26
Isoleucine	28	40	49
Leucine	66	70	82
Lysine	58	55	63
Methionine + cystine	25	35	26
Phenylalanine + tyrosine	63	60	90
Threonine	34	40	38
Tryptophan	11	10	14
Valine	35	50	50

[a] From FAO/WHO/UNU,[11] based on the recommended intake shown in Table 1 and a safe level of protein intake of 1.10 g reference protein per kilogram per day.
[b] Source: Protein Technologies International, St. Louis, MO.
[c] Histidine requirement not measured in preschool children. Value in parentheses was interpolated from data in infants and estimates in adults.

This pattern, expressed in terms of milligrams of amino acid per gram of protein, has become the standard of comparison to assess the protein quality of a food by calculating its amino acid score (according to the most limiting amino acid, i.e., the one with the largest deficit) and correcting it by the digestibility of the protein.[11] A protein with an amino acid composition that coincides with or surpasses the amino acid pattern for preschool children also compares favorably with the patterns derived from amino acid requirements of older children and adults.[11] Thus, it is the most widely used standard to assess the amino acid quality of a protein food, except for young infants.

As Table 2 shows, the current (1985) pattern of amino acid requirements has lower values for isoleucine, methionine + cystine, threonine, and valine than the provisional pattern proposed in 1973. Therefore, it has had important effects on the outcome of evaluating foods and diets in terms of their protein quality. The fact that the methionine + cystine requirements are less than what was previously estimated indicates that legumes, and particularly soy, can play an important nutritional role either as the main proteins in the diet or to supplement and improve the amino acid content and balance of foods and meals.

IV. NUTRITIONAL QUALITY OF SOY PROTEINS

Isolated soy proteins are relatively new protein sources. They were initially used in the 1960s in liquid formulas for babies intolerant to milk. Methionine was added to fortify the formulas in order to satisfy the estimated requirements of infants.

In view of the new knowledge about methionine + cystine requirements and on the amino acid composition of soy protein (Table 2), several studies were done to determine the nutritional quality for preschool children of isolated soy protein without amino acid fortification.

V. EXPERIMENTAL DESIGN AND METHODS

Nitrogen (N) balance techniques were used to estimate the protein value of the isolated soy proteins.[12,13] Regression analysis was used to calculate regression coefficients between N intake or absorption and N retention of the test proteins, and were compared with those of milk as a reference protein. The regression coefficient of intake on retention provides an estimate which is similar to net protein utilization.[13] The regression coefficient of absorption on retention is similar to biological value.[13] The higher the regression coefficient, the better is the quality or value of the protein.[13,14] Apparent nitrogen digestibility was calculated and compared with that of milk protein as a reference. "True" digestibility was calculated from the data on apparent digestibility, assuming an obligatory fecal nitrogen loss of 20 mg/kg/d.[9]

Three separate studies with preschool-age children were carried out using three isolated soy proteins at INCAP.[10-15] An additional study was conducted at the Chilean Institute of Nutrition and Food Technology (INTA) with a fourth isolated soy protein.[16] The isolated soy proteins initially identified as Supro® 620, Supro® 710, and Purina® Protein 220 were evaluated at INCAP, and SPF-200 was tested at INTA. Equivalent products are now identified as Supro® 710, Supro® 1751, Supro® 1711 from Supro® 710; Supro® 500E, ProPlus 500F, Supro® 620, Supro® 1500, and Supro® 760 from Supro® 620; PP220 from Purina® Protein 220; and Supro® 200G, ProPlus 200F, ProPlus 200FC from SPF-200. The basic process of producing these products remains unchanged and they differ only in brand name, cation used to neutralize the protein (sodium, potassium), and nutrient fortification to meet specific food requirements or microbiological specifications.

In the first study,[15] nitrogen intake levels ranging from 320 to 120 mg/kg/d (equivalent to 2.0 to 0.75 g protein, based on N × 6.25) were tested in descending order at 9-d intervals. The first 5 d in each period were for stabilization at the new level of N intake, and metabolic balance studies were done in the last 4 d. All diets supplied 100 kcal/kg/d and were supplemented with vitamins and minerals to meet the requirements of preschool children. Subsequent studies[10,15] evaluated N intakes in the range of 200 to 80 mg N per kilogram per day (1.25 to 0.5 g soy protein, N × 6.25), since it was apparent that higher intakes of soy proteins largely exceeded requirements and could not be used in the calculation of regression coefficients.[15] Either a descending order of N intake[15] or a combination of ascending and descending designs[10] were used. In the study with Supro® 710,[15] the effect of choline supplementation was studied using soy protein formulas with or without the addition

TABLE 3
Composition of a Typical Liquid Diet to Supply 1.25 g Protein Per Kilogram Per Day

Component	Amount (g)
Isolated soy protein	1.44[a]
Cornstarch	1.50[a]
Sugar	15.00[a]
Cottonseed or peanut oil	3.26
Mineral mixture[b]	0.61
Water, to total of	80.00

[a] Changes in protein intake were accompanied by equivalent isoenergetic changes in cornstarch or sugar.
[b] Provides (in meq): K, 6; Na, 1; Ca, 0.5; Mg, 0.2; Cl, 6; PO_4, 0.5; CO_3, 1; SO_4, 0.2.

of 50 mg choline chloride daily, which is equivalent to the amount of free choline present in about 500 ml of cow milk.[17] This was done based on reports which suggest that, at least for other animal species, the need for choline may increase when methionine is the limiting amino acid.[18-20]

These studies were with children in the age range of 19 to 44 months. The children were housed in the INCAP Clinical Research Center under 24-h supervision. They had been originally admitted to the Center due to malnutrition, but they had recovered completely at least 1 month prior to the initiation of the studies with soy proteins. The children were given the test meals as liquid formulas using refined diets (Table 3). Changes in protein concentration were compensated with isoenergetic changes in carbohydrates. When milk protein was evaluated, skim milk replaced isolated soy protein and sugar in isonitrogenous and isoenergetic amounts. The diets were fed as five identical daily meals. Vitamin and mineral supplements were given daily to meet the child's requirements.

The study of Egana et al.[16] used the same basic procedures with some slight modifications. The children were slightly older (31 to 62 months) and were transferred from a local orphanage to the INTA Clinical Center. The diets were a combination of liquid and solid food prepared from refined ingredients similar to those used in the INCAP studies. The nitrogen intake levels ranged from 240 to 120 mg/kg/d to provide protein levels of 1.5 to 0.75 g/kg/d (N × 6.25). The feeding period was 8 d for each protein, with 4 d for stabilization and the last 4 to measure nitrogen balance.

VI. RESULTS AND DISCUSSION

The overall acceptance of the isolated soy protein was good in all the studies. No unusual clinical changes were observed in the children by the medical and paramedical staffs nor in the biochemical parameters measured during the course of the studies.

The range of nitrogen intakes varied from 80 to 320 mg·kg/d, which gave considerable differences in apparent digestibility values.[10,15,16] However, when the apparent digestibilities were corrected for obligatory fecal nitrogen losses of 20 mg/kg/d,[9,21] the differences in digestibility due to the different intakes were largely eliminated.

The estimated "true" digestibilities of the four isolated soy proteins are summarized in Table 4. They were similar to the digestibility of milk proteins, with average values ranging from 92 to 96%. There was no effect of the level of protein intake on the estimated "true" digestibility, although there were small variations in the individual data points. The equivalency in "true" digestibility was also seen when milk and isolated soy proteins were compared in the same study.

TABLE 4
Estimated "True" Digestibility (Percent of Intake) of Isolated Soybean Protein and Milk Protein at Various Levels of Nitrogen Intake

	Isolated soy protein					Cow milk		
N intake (mg/kg/d)	Supro® 620[15]	Supro® 710[15] +Choline	Supro® 710[15] No choline	PP220[a]	SPF200[b]	Dry skim milk[a]	Dry full-fat milk[b]	Various lots[c]
320	93.2	—	—		—		—	90.2
280	95.1	—	—		—		—	90.1
240	95.3	—	—		94.5		92.8	90.3
200	98.0	95.0	96.0		93.2		88.7	93.0
160	97.0	94.5	97.5		93.4		88.9	90.5
120	98.6	95.7	96.7		88.3		91.4	—
80	—	99.0	93.0		—		—	94.0
Mean, all	96	96	96	94	92	94	90	91
Mean, 80 to 200 mg	98	96	96	94	92	94	90	93

[a] Combined data for ascending and descending study designs.[10]
[b] Recalculated from Egana et al.[16] using 20 mg N per kilogram per day as obligatory fecal N loss.
[c] Summarized by Torun[15] from other INCAP studies.[22-27]

TABLE 5
Regression Equations of Apparent Nitrogen Retention (Y) on Nitrogen Intake (X), and Estimates of Safe levels of Protein Intake

Protein source	Regression equation[a] Y = a + bX	N Intake to retain 36 mg/kg/day[b]	Safe level of protein intake, g/kg/day[c]	Ref.
Supro® 620	−56.3 + 0.65X (41.8, 0.20)	142	1.11	15
Supro® 710	−34.6 + 0.59X (17.1, 0.13)	120	0.93	15
PP220	−46.0 + 0.58X (15.0, 0.09)	141	1.10	10
SPF-200	−54.0 + 0.49X (9.4, 0.10)	184	1.43	16
Milk	−45.0 + 0.70X (22.0, 0.12)	116	0.90	10
Milk	−33.6 + 0.51X (16.2, 0.08)	136	1.07	16
Milk	−53.8 + 0.64X	140	1.10	22—27

[a] Mean of individual data on the intercepts (a) and regression coefficients (b), with corresponding standard deviations in parentheses.
[b] 26 and 10 mg N per kilogram per day for growth and miscellaneous losses, respectively.[11]
[c] N × 6.25 plus twice the coefficient of variation of 12.5%.[11]

Table 4 also shows that the addition of choline to the soy protein had no effect on digestibility.

Table 5 shows the regression equations of apparent nitrogen retention (i.e., without including sweat and miscellaneous losses) on nitrogen intake for the four isolated soy proteins and cow milk from different studies. The amounts of dietary nitrogen required to satisfy the needs of these preschool children were calculated from the equations, in order to allow a retention of 26 mg N per kilogram per day for growth of children 2 to 4 years old and 10 mg N per kilogram per day for sweat and miscellaneous losses.[11] Those amounts were then converted to protein, using a factor of 6.25, and 25% more was added corresponding to twice the population coefficients of variation to calculate the safe levels of protein intake.[11]

The safe levels of intake of the isolated soy proteins coincided with or were lower than current protein recommendations for children of this age group,[11] except for the Chilean study, which suggested the need of 0.3 g more soy than milk protein per kilogram per day.[16] On the average, the safe level of intake reported for milk by these investigators (1.02 g/kg/d) was 11% lower than the average value of 1.14 g/kg/d calculated for the four soy protein isolates.

Comparing the regression coefficients shown in Table 5 for the soy proteins and milk, the quality of the former ranged from 83 to 96% (average: 93%) in relation to milk. As was the case for digestibility, the addition of choline did not influence nitrogen retention, protein quality estimated from the regression equations, nor the safe level of soy protein intake.[15]

Table 6 shows the plasma protein concentrations after 9 d with each level of protein intake in the Guatemalan studies. Decreases were observed with intakes of 0.75 g soy or 0.5 g milk protein per kilogram per day. No differences with either soy or milk were observed between 1.5 and 0.75 g/kg/d in the Chilean study.[16,28] This suggests that, at least for the short duration of each level of intake, there were no deleterious effects with the soy isolates eaten at or below the recommended safe levels of protein intake.

VII. CONCLUSIONS

These studies showed that isolated soy proteins have a high digestibility. Their nutritional quality, although somewhat lower than that of milk proteins, is good enough to sustain adequate nitrogen balance when provided in the amounts recommended internationally for children of preschool age. These results and their essential amino acid pattern support the use of isolated soy proteins as the main protein in the diet and for blending with other foods to increase the protein supply of the diet and/or improve the quality of protein sources that are limited by their content of certain essential amino acids.

TABLE 6
Plasma Protein Concentrations (g/dl) with Different Levels of Protein Intake (mean / SD)

Protein (or N) intake (g or mg/kg/d)	Supro® 620	Supro® 710 + choline	Supro® 710	PP220		Milk	
				Descending	Ascending	Descending	Ascending
2.00 (320)	6.9 / 0.2			6.9 + 0.2		6.9 + 0.2	
1.75 (280)	7.0 / 0.3						
1.50 (240)	6.9 / 0.3				7.0 / 0.4		6.9 / .03
1.25 (200)	6.8 / 0.3	6.5 / 0.3	6.8 / 0.4	6.8 / 0.4	6.8 / 0.5	6.8 / 0.3	6.7 / 0.2
1.00 (160)	6.6 / 0.4	6.4 / 0.3	6.4 / 0.2	6.6 / 0.2	6.3 / 0.6	6.9 / 0.5	6.6 / 0.7
0.75 (120)	6.4 / 0.5[a]	6.3 / 0.5	6.3 / 0.3[b]	6.3 / 0.2[b,c]	6.2 / 0.4[b]	6.6 / 0.3	6.8 / 1.0
0.50 (80)		6.0 / 0.4[a]	5.9 / 0.3[a]	6.2 / 0.6[b]	6.2 / 0.4[b,c]	6.4 / 0.4[b]	6.5 / 0.3[b,c]

[a] Less than other levels of intake in same column, $p < 0.05$ (Student's paired t)
[b] Less than protein intake of 1.25 g/kg/d, $p < 0.01$ (Student's paired t)
[c] Different from preceding protein level. In the "ascending" design, 2 g/kg/d preceded the intake of 0.50 g/kg/d.

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