EFFECT OF DIETARY PROTEIN (MIXED, DEFATTED BEEF OR COTTONSEED) ON THE AMINO ACID STATUS OF HEALTHY ELDERLY WOMEN

A DISSERTATION

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BY

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CHAPTER I

INTRODUCTION

Because of the high protein content (approximately 60%), properly processed cottonseed has a great potential as food for humans (1). Children have demonstrated adequate nutritional status after six months of consuming diets supplemented with cottonseed protein (2, 3). In young adult women, cottonseed protein has been found to maintain nitrogen balance at 0.106 g nitrogen per kg body weight (4). Bucko-Monroe and Thomas (5) demonstrated that calcium status of young women was not affected by a diet in which cottonseed flour supplied 99% of the protein. Although there has been research into the use of cottonseed as a source of dietary protein, most has been done with young college age women and children. Little has been done to determine the effects of cottonseed flour as a protein source on the elderly.

Advances in medical care have led to an ever increasing population of elderly people. With this increase in numbers is a concomitant need for information pertaining to specific requirements associated with aging. The health and longevity of the elderly are affected by physiological, pathological, psychologic, economic, sociologic, and physical factors. These factors in turn, affect food choices and habits in the elderly (6).

The established recommended dietary allowance of protein for adults remains the same for men from age fifteen and for women from age nineteen (7). The planning of nutritionally adequate diets for older people is based largely on the extrapolation of data from studies with healthy young adults (6). Muscle accounts for over 50% of the total body pool of free amino acids (8). Muscle mass decreases with age, but the affect this decline has on total body amino acid metabolism and dietary requirements for amino acids is not known.

The nutritional attributes of a protein can be evaluated in many ways. Results of studies examining the relationship between dietary intake and plasma amino acid levels are conflicting. Nonetheless, plasma amino acid profiles remain one of the more acceptable and widely used means of assessment. Most investigations have utilized unusually high or unusually low intakes of protein and monitored postprandial plasma levels. Few of the studies included the elderly.

The growing interest in the use of cottonseed as a protein source, the limited research in this area with the elderly, and the need for identification of any alterations in amino acid status of the elderly incident to change in dietary protein, led to the initiation of this study. The purpose of this study was to determine

the comparative effects of beef versus cottonseed protein on the amino acid status of healthy elderly women as reflected in the plasma and urinary amino acid profiles.

The following were the specific objectives of this investigation:

1. To analyze and compare the following amino acids in plasma for each dietary period i.e., mixed protein, defatted beef, and cottonseed protein; arginine, leucine, threonine, alanine, citrulline, glycine, histidine, isoleucine, lysine, methionine, ornithine, phenylalanine, proline, serine, taurine, tyrosine, valine, glutamine, and phosphoethanolamine
2. To analyze and compare the following amino acids in urine during each dietary period: phosphoserine, alanine, glycine, 3-methylhistidine, phosphoethanolamine, cysteine, histidine, taurine, leucine, threonine, methionine, serine, and cystathionine
3. To calculate and compare the nonessential to essential amino acid ratios during each dietary period in the plasma using the formula:

$$NE:E = \frac{\text{glycine+serine+glutamine+taurine}}{\text{leucine+isoleucine+valine+methionine}}$$

4. To compare the effects of dietary source of protein on plasma and urinary amino acids when the amino acids are considered on the basis of structure and/or function

CHAPTER II

REVIEW OF LITERATURE

Physiologic Changes with Aging and the Effects on Protein Metabolism

In man, aging is so modified by disease that its true course is unknown (9). Chronic diseases occur with increasing frequency as aging progresses. The role of long term nutrient intake in pathogenesis of these diseases (such as osteoporosis, diabetes, hypertension, and atherosclerosis) remain uncertain (7). There are changes that occur on the molecular level with aging that are associated with both structural and functional modification in most organ systems (10).

Reductions in organ system functions are commonly found with aging. These organ systems are not affected uniformly. Howell and Loeb (11) grouped changes according to their effect on nutritional status: (a) interference with nutrient intake, (b) interference with absorption, storage, and utilization of nutrients, and (c) an increase in excretion and need for specific nutrients. Contributing physiologic changes include reduced motility of the stomach, a reduction in the peristaltic activity of the intestine and colon, a gradual loss of functioning nephrons, and a decrease in activity/turnover of several digestive and metabolic enzymes (11, 12, 13).

These alterations alone could affect the efficiency of absorption, transport, and excretion of nutrients in general, and proteins and amino acids in particular. The practical effects of these changes on nutritional status have been discussed and questioned (14).

A pattern of decreased activity has been demonstrated in mature mammals with increasing body size for cellular and sub-cellular aspects of protein metabolism such as plasma albumin synthesis, liver RNA content, and enzyme activity. This pattern has been extrapolated to aging man and reviewed extensively by Munro (15).

More recently, Young (6, 16) demonstrated that the loss of muscle mass accompanying aging is associated with a 63% reduction in the protein turnover as compared to young adults, when expressed in terms of body weight. An increase in protein turnover was found when expressed per g creatinine excretion. These findings were interpreted to indicate that there is a shift in the distribution of body protein synthesis with aging. The shift is directed toward active visceral tissues relative to whole body protein metabolism, and is accompanied by a decline in both the rate of muscle protein breakdown and synthesis in the elderly subject (6). These findings have been supported by studies examining the comparative excretion of 3-methylhistidine in young adults and the elderly (17). Long, et

al (18) demonstrated that the measurement of urinary 3-methylhistidine is a valid measure of the amount of 3-methylhistidine released by protein breakdown and can be used as a procedure for evaluating protein turnover in man. Using data on the urinary output of 3-methylhistidine and the estimated protein-bound 3-methylhistidine in subjects' muscle tissue, the daily breakdown of skeletal muscle protein can be computed (19). Protein metabolism and requirements for the elderly may differ from that of young adults, whether one is considering the process of aging itself or the physiological changes and diseases associated with it.

Regulation of Plasma Amino Acids

Plasma amino acid concentrations reflect the balance of their inflow (dietary intake, rate of gastric emptying, intestinal absorption, reabsorption in the kidney, release from endogenous protein stores, and net endogenous synthesis of nonessential amino acids), and their outflow (uptake into the liver and nonhepatic tissues, endogenous protein synthesis, and catabolism) (20, 21). Protein consumed in the diet is enzymatically hydrolyzed in the stomach and small intestine into small peptides and amino acids. These are combined with endogenous proteins supplied by intestinal secretions and cells shed from the mucosa. The amino acids are transported across the epithelial cells of the intestinal mucosa,

enter the blood via the portal vein, and combine with amino acids coming from the tissues to form the plasma amino acid pool.

Competitive inhibition of intestinal absorption exists between amino acids (22). Intestinal perfusion experiments with man have shown that the absolute amount of the individual amino acids absorbed from a mixture depends directly upon its amino acid composition in terms of molar ratios. More specifically, when valine is perfused with a mixture of amino acids saturation occurs at 10 mM concentration, whereas perfusion of valine alone is marked by the absence of saturation at 60 mM concentration. The intestinal absorption of amino acids is sodium-dependent (23) and takes place at a faster rate when in the form of small peptides (24).

The plasma amino acid pool, though small compared to the total body pool, is in rapid flux with the extravascular pools and may be a sensitive indicator of the state of depletion or repletion (25). The fact that plasma amino acid levels fluctuate less than 50% despite dietary intakes tenfold greater than the amounts of amino acids initially present in the extracellular space, further implicates the importance of tissue uptake and metabolism in the control of amino acid levels in plasma (21). The interorgan exchange of amino acids via the plasma has been reviewed by Felig (26).

Since the classic work of Schoenheimer, Ratner, and Rittenberg (27), the fact that proteins and amino acids are in a dynamic equilibrium in mammals has been accepted. The dynamic state of protein and amino acid metabolism has been reviewed by Albanese and Orto (28) and Longnecker (29). A schematic representation of this dynamic state is shown in Figure 1.

The body attempts to control the total concentration of blood amino acids at a steady-state within relatively narrow limits at the expense of one or more individual components (30). The metabolism of certain amino acids therefore, can be altered or tissue concentrations reduced without affecting nitrogen balance.

Within individuals the regulatory mechanisms that control variations of amino acids in plasma are partially dependent on circadian rhythms (31), nutritional factors (20), age (32), and sex (33) of a healthy individual. Between individuals, normal variation in plasma concentration of amino acids is usually defined either in terms of the fasting mean value $\pm$ one standard deviation or as the mean value and range of data (24). Recognition of such factors is important if aberrations in plasma amino acid levels are to be recognized.

Plasma Amino Acid Profiles of the Elderly

Normally there is an extensive cellular reutilization of the amino acids released during the course of protein breakdown. If these rates of synthesis and breakdown change, or the degree of

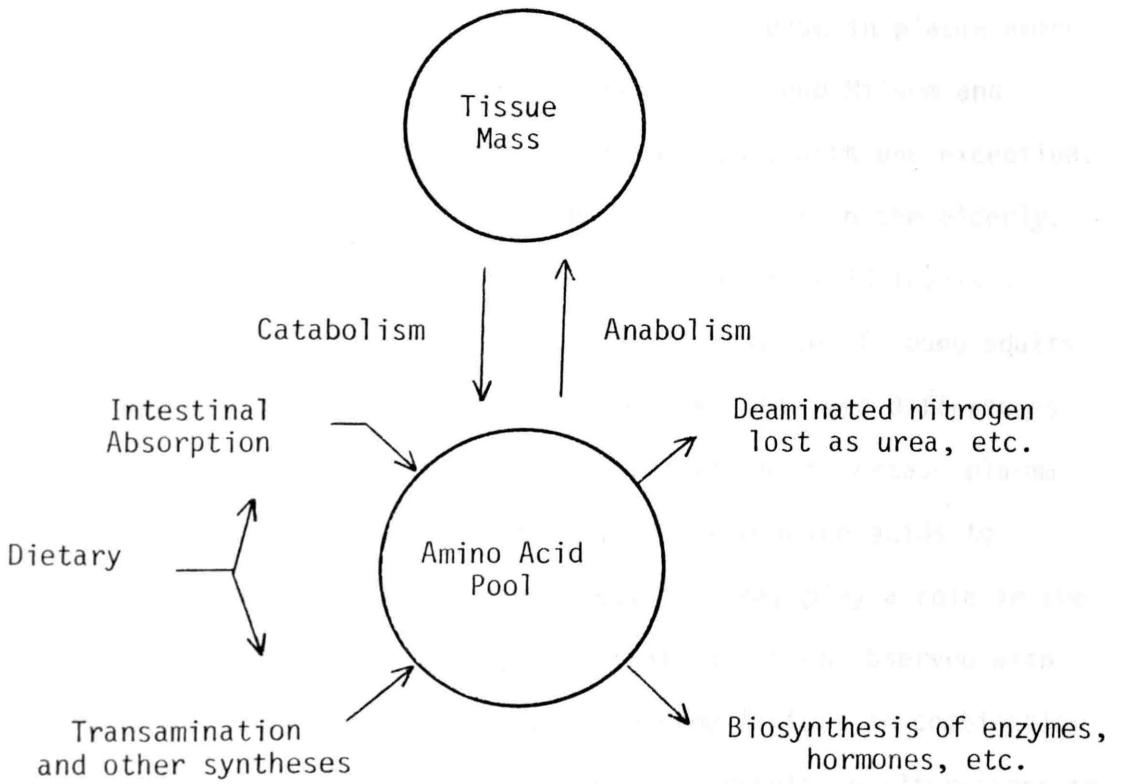

Figure 1. Dynamic equilibrium state of protein metabolism (28).

amino acid reutilization is altered as aging progresses (6), this would be expected to be reflected in the plasma amino acid profile.

The literature is at variance as to alterations that occur in the amino acid profile with aging (32, 33, 34, 35, 36). Bertolini (34) and Galante (33) found an increase in plasma amino acids in women with age. Wehr and Lewis (32) and Milsom and co-workers (36) found no differences with age, with one exception. Wehr and Lewis (32) found ornithine to be higher in the elderly. Ackermann and Kheim (35) found the plasma amino acid levels of elderly subjects to be generally lower than those of young adults.

Bertolini (34) has suggested that the observed differences between males and females in the concentration of certain plasma amino acids, reflect the sensitivity of these amino acids to endocrine regulation. Endocrine regulation may play a role in the changes of plasma amino acid levels that have been observed with aging, especially among females. Endocrine factors in combination with the intrinsic phenomena of aging, may result in alterations in fasting plasma amino acid levels. It is appropriate then, to group subjects according to both age and sex when studying plasma amino acid levels.

Effect of Diet on Plasma Amino Acids

Results from numerous studies examining the relationship between dietary intake and plasma amino acid levels have been

conflicting (6, 20, 25). Most have used unusally high or unusually low levels of protein intake and have measured postprandial plasma levels of amino acids.

McLaughlin (37) has stated that a moderate deficiency of an amino acid in the diet of a growing animal leads to an inordinately low concentration of that amino acid in the plasma. McLaughlin concluded that, although there is usually a good correlation between the amount of an amino acid in plasma and in the proteins fed, other dietary constituents may influence plasma amino acid concentrations.

Harper (20) observed that dietary amino acid imbalances cause changes in plasma amino acids within a short time after ingestion. The changes persist for several hours after the meal and seem to be reinforced by consecutive meals. McCarver (38) found that essential amino acid patterns in the postprandial plasma of elderly subjects paralleled those found in the cottonseed formula consumed. Sneed (39) found that fasting plasma amino acid concentrations of lysine reflected the dietary intakes more reliably than for any other amino acid. The significance of diet with regard to plasma amino acids has been reviewed by Young and Scrimshaw (40).

Swenseid (25) found that, in adult human subjects, levels of some amino acids in plasma change with dietary intake, while others remain relatively constant. Swenseid has suggested that the amino acids required in the diet for maintenance of nitrogen equilibrium could be divided into two categories on this basis. Several liver

enzyme activities involved in the catabolism of the essential amino acids are responsive to alterations in dietary intake (20, 25). One possible explanation is that, for some amino acids, the alterations in the catabolic rates that might be associated with different amounts of dietary protein or amino acids may occur rapidly and be of sufficient magnitude to maintain relatively constant levels of these amino acids in plasma (25). The question of whether ability to accommodate to alterations in amino acid intake is affected by age remains to be answered.

Plasma Amino Acid Ratios

As an alternative to examining absolute values of amino acids, some investigators have chosen to look at amino acid ratios in the plasma. Whitehead and Dean (41) have proposed that a ratio of nonessential to essential amino acids in a fasting plasma sample is a reliable index of a child's nutritional status, using the equation:

$$NE:E = \frac{\text{glycine+serine+glutamine+taurine}}{\text{leucine+isoleucine+valine+methionine}}$$

An index of 2.0 or below is indicative of optimum nutrition, whereas a ratio above 4.0 is associated with clear signs of malnutrition. Using this equation, in a study of young adult women, Sneed (39) also found an increasing index as protein intake decreased. At the lowest intake of 7.0% of kilocalories, the average ratio was 3.28.

Fernstrom and others (42) examined plasma amino acid ratios in relation to varying amounts of dietary protein and found that the ratio of plasma tryptophan, tyrosine, or phenylalanine to the sum of other large neutral amino acids fell as the protein content of the diet increased. The group of neutral amino acids included tyrosine, tryptophan, phenylalanine, isoleucine, valine, methionine, and leucine. Conversely, the ratio of valine to the sum of neutrals increased as protein was added to the diet. There was no correlation between dietary protein and ratios for either leucine or isoleucine.

Anderson and Blendis (43) found that plasma tryptophan/neutral amino acid ratios correlated with both long term and immediate quantity of protein consumed. The plasma tyrosine/neutral amino acid ratios correlated with the long term energy consumption of individuals. No correlations were found using tryptophan/branched-chain amino acids or acidic amino acids/branched-chain amino acids.

Structurally and Functionally Related Amino Acids

Most information in the literature concerned with plasma amino acids according to their structural or functional groupings has been in the area of inborn errors of metabolism. The topic of inborn errors of metabolism is beyond the scope of this study and will not be reviewed here.

There are various ways for categorizing amino acids. For the purpose of this study, amino acids will be grouped as follows:

1. Neutral
2. Basic
3. Branched-chain
4. Aromatic
5. Hydroxylated
6. Urea cycle

As a group, the most widely studied amino acids in recent years have been the branched-chain amino acids. The branched-chain amino acids are elevated in diabetes and obesity (47) and are abnormally low in chronic liver disease (48). Fernstrom and others (42) found that plasma concentrations of leucine, isoleucine, and valine each varied directly with the quantity of protein in the diet. In addition to branched-chain amino acids, Fernstrom and others (42) found that the aromatic amino acids phenylalanine, tyrosine, and tryptophan also varied during the day with the amount of protein in the diet. A similar pattern was seen for methionine. The smaller molecular weight neutral amino acids glycine and alanine varied inversely with dietary protein content.

The comparison of fasting plasma amino acids in young adult females while consuming mixed protein, cottonseed protein, or beef protein diet revealed some differences (49). The concentrations of branched-chain and neutral amino acids were significantly higher during the period in which the protein was obtained from a mixed diet. No differences in plasma concentrations of the sulphur containing amino acids, cystine, and methionine incident to dietary source of protein were found.

Regulation of Urinary Amino Acids

Urinary loss of free amino acids is principally the result of a balance between filtered load and tubular resorption (44). The pattern and concentration of amino acids present initially in the glomerular filtrate and the efficiency of the absorptive process modulate the composition of the final urine (24). Between certain individual as well as groups of amino acids, there exists competition for reabsorption in the tubules (45). Four distinct amino acid transport systems have been classified within the mammalian kidney (46):

1. The neutral system
2. The basic system
3. The acidic system
4. The iminoglycine system

These competing transport systems could be responsible for changes in the excretion of individual amino acids with no apparent changes in nitrogen balance.

Amino acids are actively absorbed through the tubular epithelial cells of the proximal tubule by means of a "symport" or simultaneous transport of two compounds in one direction (50, 51). The amino acids are transported from the tubular lumen through the brush border by combining with a carrier molecule. A sodium ion also combines with the carrier. The passage of sodium down its concentration gradient causes simultaneous transport of amino acids against their concentration gradient (50). In certain animal tissues glutathione has been postulated as the carrier molecule

after the amino acid has been bound to a specific membrane site through a membrane-associated protein (52).

In the normal adult, only about 2 to 3% of the total urinary nitrogen in bladder urine is accounted for by amino acids, and this fraction represents less than 5% of the filtered amino acid load (24). When the filtered loads are increased, the amounts of amino acid both excreted and reabsorbed are increased (45). The literature on the urinary excretion of amino acids prior to 1970 has been extensively reviewed by Albanese and Orto (54).

Tweksbury and Lohrez (53) studied the circadian nature of amino acid excretion and suggested that a basic rhythm existed under the control of endogenous factors and that the effects of ingestion of food are added to the basic rhythm. The basic rhythm is characterized by maximum excretion value at mid-morning and minimum excretion value in the early morning hours.

Urinary Amino Acid Profile

Diet, age, sex, and physiological status, as well as disease processes, each account for some of the variation found in urinary amino acid profiles (24). There is, however, considerable variation in the excretion of free amino acids, even in an apparently homogenous group of normal individuals.

There are great discrepancies in methods of collection and expressions of concentration for urinary amino acids making survey comparisons difficult. Urinary amino acid concentrations have been

reported from collections of four hour urines from fasting subjects (55) as opposed to 24-hour urines from non-fasting subjects. The latter is the usual and preferred procedure. The values obtained have been expressed as ug/mg creatinine (55), um/g creatinine/3 hours (53), um/24 hours (56), and mg/24 hours (57).

Soupart (58) estimated that glycine, taurine, histidine, 1-methylhistidine, 3-methylhistidine, glutamine, serine, alanine, and amino butyric acid, in descending order, account for 85% of the total free amino acid excretion. Stein (59) ten years earlier had found that 70% of the amino acids determined in a 24-hour sample could be accounted for by taurine, glycine, histidine, and methyl-histidine. Aspartic acid, glutamic acid, proline, methionine, and arginine are virtually absent (10-15 mg per day) in a freshly voided sample from a normal subject (40, 59).

A recognized age difference exists for urinary amino acid excretion between normal newborns and normal adults (58). The newborn has a higher rate of urinary amino acid excretion relative to total excretory nitrogen. During the first few years of life, the amino acid pattern of the urine changes toward that of an adult (40). The higher clearance rates in infancy reflect lower rates of tubular reabsorption in this age group. Changes in the specific activities of various transport sites during development and an increase in the absorptive area with maturation, explain, in part, the age-dependence of aminoacidurias. As there is a loss of

functioning nephrons with age, it is possible that differences exist in the urinary amino acid profile between the young adult and aged adult.

Effect of Diet on Urinary Amino Acids

The possible relationship between nutritional value of dietary protein and urinary amino acid levels has not been so extensively investigated as that of plasma levels. The factors affecting free amino acid excretion in normal healthy adults have been listed by Soupart (58) as: (1) menstrual cycle, (2) normal pregnancy, (3) hormonal factors, and (4) high protein diets. Although Soupart (58) listed high protein intakes as a factor, Soupart added that the free amino acid excretion is poorly influenced by a supply of up to 4 g protein/kg of body weight per day.

Block and others (60), using two human subjects, and Stein (61) using one subject, found that the amino acid composition of urine in normal individuals was relatively insensitive to the amount of protein in the diet. Stein and others (60) reported that a 13-fold increase in dietary protein resulted in a 2 to 3-fold increase in the urinary excretion of most amino acids. Block (61) administered an additional 9 g of free L-methionine at two levels of protein intake. This resulted in an increase in urinary methionine, but the excretion did not exceed 1% of the methionine ingested.

Adibi (44) examined urinary amino acids in obese and normal subjects during short term starvation and isocaloric, protein-free

feeding. The results indicated no differences in the excretion of eight amino acids studied between the fasted and protein-deprived subjects. There was however, a significant difference in the excretion rates of six amino acids. Leucine, isoleucine, valine, and amino-isobutyric acid were lower in the protein deprived group as compared to the starved group; whereas, taurine and alanine were higher in comparison. Adibi (44) concluded that the difference in excretion patterns of free amino acids was principally a reflection of differences in plasma amino acid composition during these altered nutritional states. A hyperaminoaciduria similar to that seen in the starved group is associated with protein-calorie malnutrition (40).

Nasset and Tulley (62) found no correlation between the biological value of ingested protein and the total amount of essential amino acids excreted in the urine. Microbiological techniques were employed in their investigation as the automated chromatographic procedures used today were not available. In his review of urinary nitrogen metabolites, Kiriyama (63) stated, "It may be said with reservation that in spite of many investigations, we have not obtained conclusive data to suggest the definite correlation between the amino acid excretion pattern and the quality and quantity of dietary protein."

However, some investigators have observed differences. Anderson and Linkswiler (56) administered casein and amino acid

diets to young men where the nonessential nitrogen was furnished by different sources. Several differences in amino acid excretion were found, with the tendency for total amino acid excretion to be lower when casein was given. The excretion ratio of some amino acids to intake of that amino acid has also been found to reflect the state of protein intake (64).

CHAPTER III

METHODS AND PROCEDURES

The present study investigated the comparative effects of dietary cottonseed or defatted beef protein on the plasma and urinary acid profiles of healthy elderly women when each provided 90% of dietary protein, respectively. All procedures in the present study were approved by the Texas Woman's University Human Research Review Committee.

Subjects

Ten healthy elderly women 65-93 years of age participated in the twelve-week study. All subjects were local Denton, Texas residents. Eight subjects lived at home and two were residents of Fairhaven Nursing Home in Denton. Each subject was examined by a licensed physician and determined to be in good health, capable of having blood drawn, and to be without disease. All subjects were non-smokers. The age, height, weight, and calculated caloric requirements for each participant are given in Table I. Biochemical parameters of protein status are given in Table II. Subjective selection was based on interest, motivation, and understanding of experimental protocol.

TABLE I

AGE, HEIGHT, WEIGHT, AND CALORIC REQUIREMENTS OF EACH PARTICIPANT[1]

Subject	Age (Years)	Height (Centimeters)	Weight (Kilograms)	Energy Requirement (Kcal)	(Kcal/Kg)	Kcal as Protein %
3441	87	157.5	58.41	1597	27.34	12
3442	93	147.3	42.05	1384	32.91	10
3443	87	152.4	46.36	1435	30.95	10
3444	82	163.8	75.45	1854	24.56	13
3445	72	164.5	67.27	2015	29.95	11
3446	77	165.1	69.00	1607	23.29	14
3447	65	165.1	86.14	1894	21.99	15
3448	71	154.9	77.70	1930	24.84	13
3450	78	158.8	62.95	1962	31.17	10
3451	68	154.9	80.00	1977	24.71	13

[1]Caloric requirements of each participant as computed by the Harris-Benedict equation (65).

TABLE II

INITIAL PROTEIN STATUS OF PARTICIPANTS AS INDICATED BY VARIOUS PARAMETERS

Subject	Plasma Total Pro. (g/dl)	Plasma Albumin (g/dl)	Plasma Globulin (g/dl)	Plasma A/G Ratio	Blood Hemoglobin (g/dl)	Blood Hematocrit (%)
3441	6.8	4.0	2.8	1.4	13.1	38.3
3442	7.2	4.3	2.9	1.5	13.1	39.3
3443	6.1	3.6	2.5	1.4	14.7	41.7
3444	6.2	3.5	2.7	1.4	13.0	38.3
3445	6.9	3.7	3.2	1.2	12.9	37.9
3446	6.8	4.1	2.7	1.5	13.3	38.9
3447	7.1	4.0	3.1	1.3	13.5	38.7
3448	6.3	3.9	2.4	1.6	13.1	38.5
3450	6.8	4.0	2.8	1.4	13.8	40.3
3451	7.0	4.1	2.9	1.4	13.9	40.9

Diets

The study of the plasma and urinary amino acid profiles involved a comparison of diets containing 90% of the protein from either glandless cottonseed flour or defatted beef. During the two equilibration periods, the subjects were provided a mixed protein diet that met individual requirements.

The glandless cottonseed flour was obtained from Texas A & M University and made into wafers that were ground into powder and combined with casserole dishes. The beef was defatted by hexane extraction (USDA Regional Research Center, New Orleans, Louisiana). The defatted beef was then baked with corn oil margarine at 350°F for ten minutes to improve palatability and combined with the same basic casserole dishes. Sample menus may be found in Appendices A and B.

Caloric requirements were determined by the Harris-Benedict Equation (65) which takes height, weight, age, and activity into account. Fat provided 35% of total kilocalories whereas protein (.8 g per kilogram body weight) and carbohydrate composed the remainder of the calories. All fat was in the form of corn oil and corn oil margarine.

All ingredients were weighed individually prior to preparation of recipes and the final product weighed after preparation. Nutrient content of the final product was then calculated on a per gram basis. Nutrient content of all other food items used were

calculated on a per gram basis using USDA Handbook #8 (66) and #456 (67). All food portions were weighed individually prior to service. Vitamin and mineral supplements given are presented in Table III. Calcium supplements were given daily in the form of calcium gluconate. Amino acid analysis of the experimental proteins are given in Table IV.

Experimental Design

The ten participants were randomly and equally divided into two groups for the duration of the study. The study consisted of four 3-week dietary periods. The first and third provided the mixed protein diet, while the second and fourth provided the experimental diets in a crossover design as diagrammed in Figure 2.

The subjects were transported three times daily to the feeding site and returned to their residence after each meal. Fasting blood samples were drawn by a qualified technician every five to six days during each dietary period. The mean of the last two consecutive blood draws for each dietary period are reported. Urine was collected on 24-hour basis and a 10% aliquot from each 24-hour volume was collected to form a five-day pool. Collection jars contained 1 ml toluene as a preservative. All samples were frozen until analyses could be performed. All analyses were run in duplicate and means were used for statistical analyses.

TABLE III

COMPOSITION OF VITAMIN AND MINERAL SUPPLEMENTS[1]

Vitamin or Mineral	Daily Intake
Vitamin A	10,000 I.U.
Vitamin D	400 I.U.
Thiamine Mononitrate	10 mg
Riboflavin	10 mg
Pyridoxine HCl	5 mg
Cyanocobalamin	5 mcg
Niacinamide	100 mg
Calcium Pantothenate	20 mg
Sodium Ascorbate	200 mg
Vitamin E	15 I.U.
Potassium Iodide	.15 mg
Iron	12 mg
Copper Sulfate	2 mg
Manganese Sulfate	1 mg
Magnesium	65 mg
Zinc Sulfate	1.5 mg

[1]Skillern's Therapeutic M, Skillern's Drug Stores, Dallas, Texas 75221.

TABLE IV

COMPARISON OF AMINO ACID CONTENT
OF GLANDLESS COTTONSEED FLOUR
AND DEFATTED BEEF

	Cottonseed Flour		Defatted Beef	
Amino Acid	g/100 g Flour	g/100 g Protein	g/100 g Flour	g/100 g Protein
Histidine	1.59	2.76	2.68	3.11
Isoleucine	1.58	2.74	4.06	4.71
Leucine	2.93	5.08	6.86	7.96
Lysine	2.35	4.07	7.22	8.38
Methionine	1.22	2.11	2.37	6.23
Phenylalanine	2.99	5.19	3.55	4.12
Threonine	1.66	2.88	3.82	4.78
Valine	2.43	4.21	4.20	4.87
Alanine	3.69	6.40	5.39	6.26
Arginine	6.58	11.42	5.44	6.31
Aspartic Acid	4.79	8.31	7.96	9.24
Glutamic Acid	10.90	18.92	13.39	15.54
Glycine	3.40	5.90	5.12	5.94
Proline	2.21	3.83	4.08	4.74
Serine	2.32	4.02	3.44	3.99
Tyrosine	1.66	2.88	2.95	3.42

Analytical Procedures

Both plasma and urinary amino acids were separated on a Beckman 121M microcolumn amino acid analyzer according to the procedures outlined by Beckman (68) based on the original work by Stein and Moore (69, 70). Blood samples, which were collected in

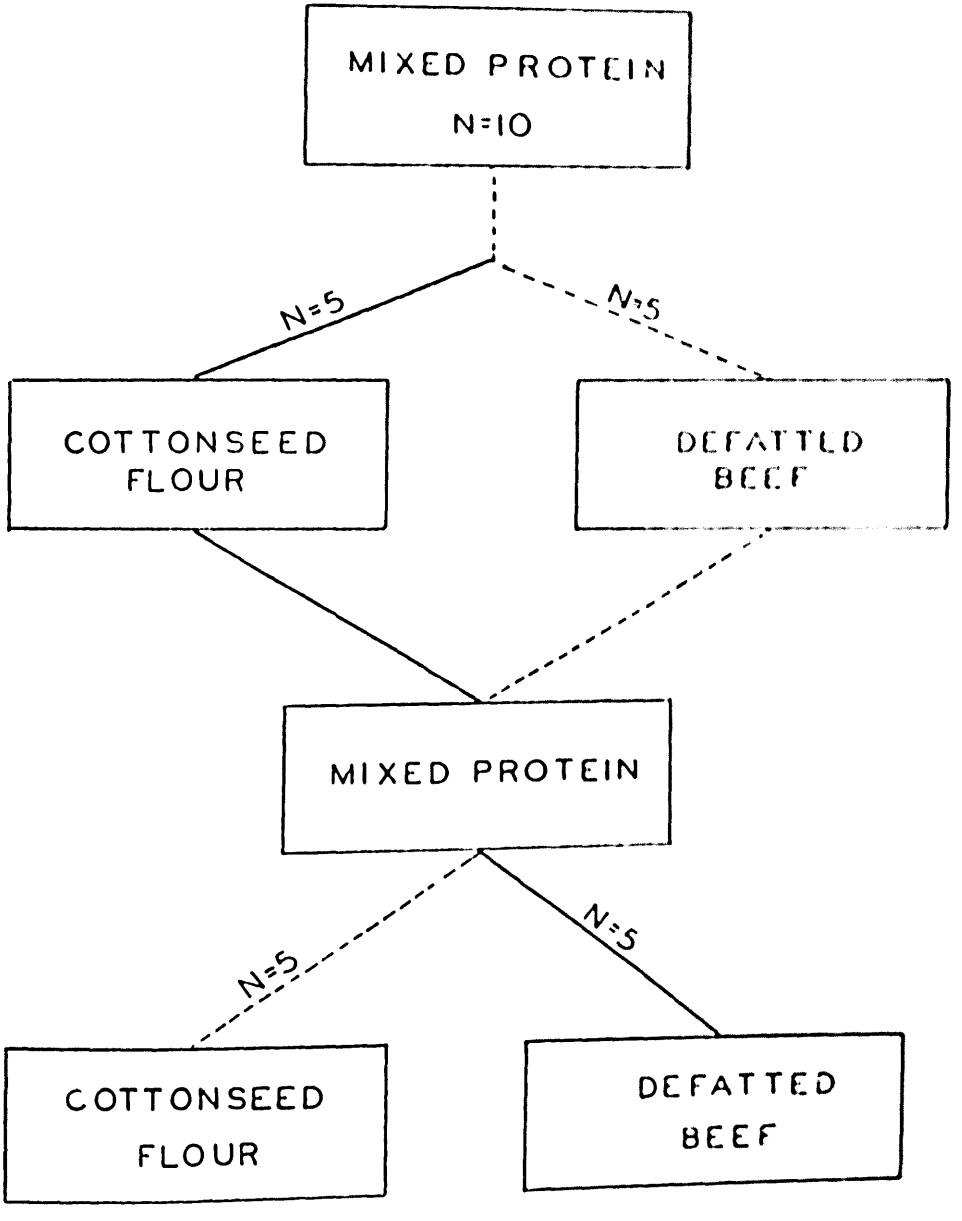

Figure 2. Dietary Protocol.

heparinized tubes, were immediately deproteinized in preparation for analysis by ion-exchange chromotography. After deproteinization of 2.0 ml of plasma with 90 mg of sulfosalicylic acid crystals, the supernatant plasma samples were diluted with an equal volume of pH 2.2 sodium citrate buffer which contained norleucine and amino guanidino propionic acid as internal standards. The samples were then passed through a filter of pore size 0.45 um. Samples were frozen until time for analyses.

A 5.0 ml portion of the five-day urine pool was adjusted to pH 2.2 using narrow range pH paper. The pH adjusted urine was then diluted 5:1 with pH 2.2 sodium citrate buffer containing the same internal standards as the plasma but in greater concentrations. The diluted sample was then passed through a filter of pore size 0.45 um and the sample frozen until time for analyses.

Statistical Analyses

A repeated measures design was applied to the mean plasma and urine values of each dietary period. Values were tested at (P 0.05) and (P 0.01) levels of significance by the Newman-Keuls' procedure for repeated measures. Simple correlation and regression were performed with the plasma and urinary values. Values are reported with standard deviation and not standard error of the mean.

CHAPTER IV

PRESENTATION AND DISCUSSION OF FINDINGS

The following is descriptive information of the amino acid profile in the plasma and urine samples of healthy, elderly women who were fed cottonseed flour or defatted beef as 90% of their protein source.

Fasting Plasma Amino Acids

Means and standard deviations for fasting plasma amino acids in the ten subjects are reported in Table VI. Aspartic acid is not reported in this study because, for most subjects, values were low, usually below analytically reliable limits. Cysteine or one-half cystine is not reported due to poor reproducibility in duplicate runs.

A comparison of fasting plasma amino acid levels previously established in elderly women (Table V), with those from the present investigation (Table VI), indicate that thirteen of the nineteen initial fasting amino acid values were lower in this study than those reported in the literature. All initial plasma amino acid values were lower than those values for the remainder of the study. The initial values for citrulline, phenylalanine, tyrosine, leucine, and alanine were significantly lower (P 0.05) than throughout the

TABLE V

CONCENTRATIONS OF AMINO ACIDS IN THE PLASMA OF FEMALES OVER 63 YEARS OF AGE FOUND IN THE LITERATURE

Amino Acid	um/100 ml		
	Ackerman (35)	Galante (33)	McCarver (38)
Threonine	14.55	14.08	12.90
Valine	21.64	21.08	20.30
Methionine	2.32	1.50	3.00
Isoleucine	6.85	5.40	5.70
Leucine	11.74	12.60	11.89
Phenylalanine	5.54	6.40	4.35
Lysine	12.37	17.70	22.11
Histidine	8.95	6.30	7.59
Aspartic Acid	2.05	3.50	-----
Serine	9.08	14.90	10.04
Glutamic Acid		-----	7.76
Proline	19.92	19.20	18.84
Citrulline	-----	4.40	-----
Glycine	20.82	32.30	30.28
Alanine			
amino butyric acid	-----	1.70	-----
Ornithine	6.32	11.70	-----
Arginine	7.05	7.70	11.73
Glutamine	-----	-----	39.85
Tyrosine	5.89	6.70	5.76

TABLE VI

MEAN CONCENTRATION OF AMINO ACIDS IN FASTING PLASMA OF ELDERLY FEMALE SUBJECTS[1]

Amino Acid	Source of Dietary Protein				
	Initial[2] (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Ornithine	7.32± 8.56[3]	6.84± 1.66	6.47± 1.80	6.46± 1.06	8.35± 2.66
Histidine	4.36± 2.44	6.38± 1.24	6.11± 2.13	5.74± 1.01	5.74± 4.36
Glycine	23.13±14.17	30.55±11.64	29.80±14.29	31.37±14.02	29.53± 9.89
Phenylalanine	2.82± 1.61	4.49± 0.65	4.28± 1.20	4.63± 0.65	3.79± 1.13
Proline	16.99±15.71	19.60±12.32	12.07± 5.72	15.29±11.23	13.50±10.62
Serine	8.30± 5.22	10.77± 2.85	9.77± 3.59	10.71± 2.25	11.13± 2.55
Taurine	4.20± 2.67	5.44± 1.37	5.64± 1.39	5.38± 1.63	5.20± 2.18
Lysine	15.65± 9.14	20.66± 8.15	22.54± 5.92	22.08± 6.25	16.50± 6.13
Methionine	1.37± 0.86	1.98± 0.49	2.12± 0.59	1.98± 0.37	2.14± 0.71
Citrulline	2.29± 1.34	4.75± 1.35	4.47± 1.22	4.86± 1.08	4.40± 1.15
Isoleucine	2.89± 1.72	4.30± 0.95	9.02±13.09	4.39± 0.93	4.48± 1.63
Tyrosine	3.55± 2.01	5.64± 1.23	5.05± 1.78	6.01± 1.31	5.46± 2.01
Valine	10.79± 6.18	16.61± 5.25	16.26± 5.83	16.79± 3.87	16.60± 8.40
Phospho-ethanolamine	1.94± 0.89	3.02± 0.90	2.37± 1.95	2.62± 0.47	2.35± 1.20

[1]Values expressed in um/100 ml.

[2]Based on sampling from nine subjects.

[3]Standard deviation.

TABLE VI-Continued

Amino Acid	Source of Dietary Protein				
	Initial (Mixed)	Equilibrated (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Arginine	8.86± 5.13	12.38± 3.81	11.77± 3.46	11.87± 2.06	13.21± 5.28
Leucine	5.98± 3.45	9.19± 2.24	8.91± 2.44	8.90± 1.47	8.56± 2.63
Threonine	7.32± 4.58	10.59± 4.58	10.01± 2.91	11.47± 2.64	8.76± 3.81
Alanine	26.44±16.87	38.21±15.07	33.47±12.89	37.78±11.60	34.42±16.09
Glutamine	18.54±16.80	53.84± 8.16	40.10± 7.83	51.91± 7.25	44.27± 8.04

remainder of the study. Values of these five amino acids for the four dietary periods were not significantly different from each other. These findings differ from those of Sneed (39), who found that most initial concentrations of amino acids in the plasma of college-age women were higher than those found during an experimental period in which 17% of the kilocalories were derived from the protein source, cottonseed flour. The concentrations of initial plasma free amino acids within the range previously established for elderly women included ornithine, glycine, lysine, and arginine. Taurine was found to be lower and phosphoethanolamine higher than values for elderly women reported by Bertolini (34).

Effect of Protein Source on Plasma Amino Acids

Swenseid (25) found that in adult human subjects, levels of some amino acids changed with dietary intake, while others remained relatively constant. McCarver (38) found that essential amino acid patterns in the postprandial plasma of elderly subjects paralleled those found in the cottonseed formula consumed. Sneed (39) found that fasting plasma amino acid concentrations of lysine reflected the dietary intakes from cottonseed flour more reliably than any other amino acid.

In the present study, changes in lysine and threonine paralleled each other (Figures 3 and 4).

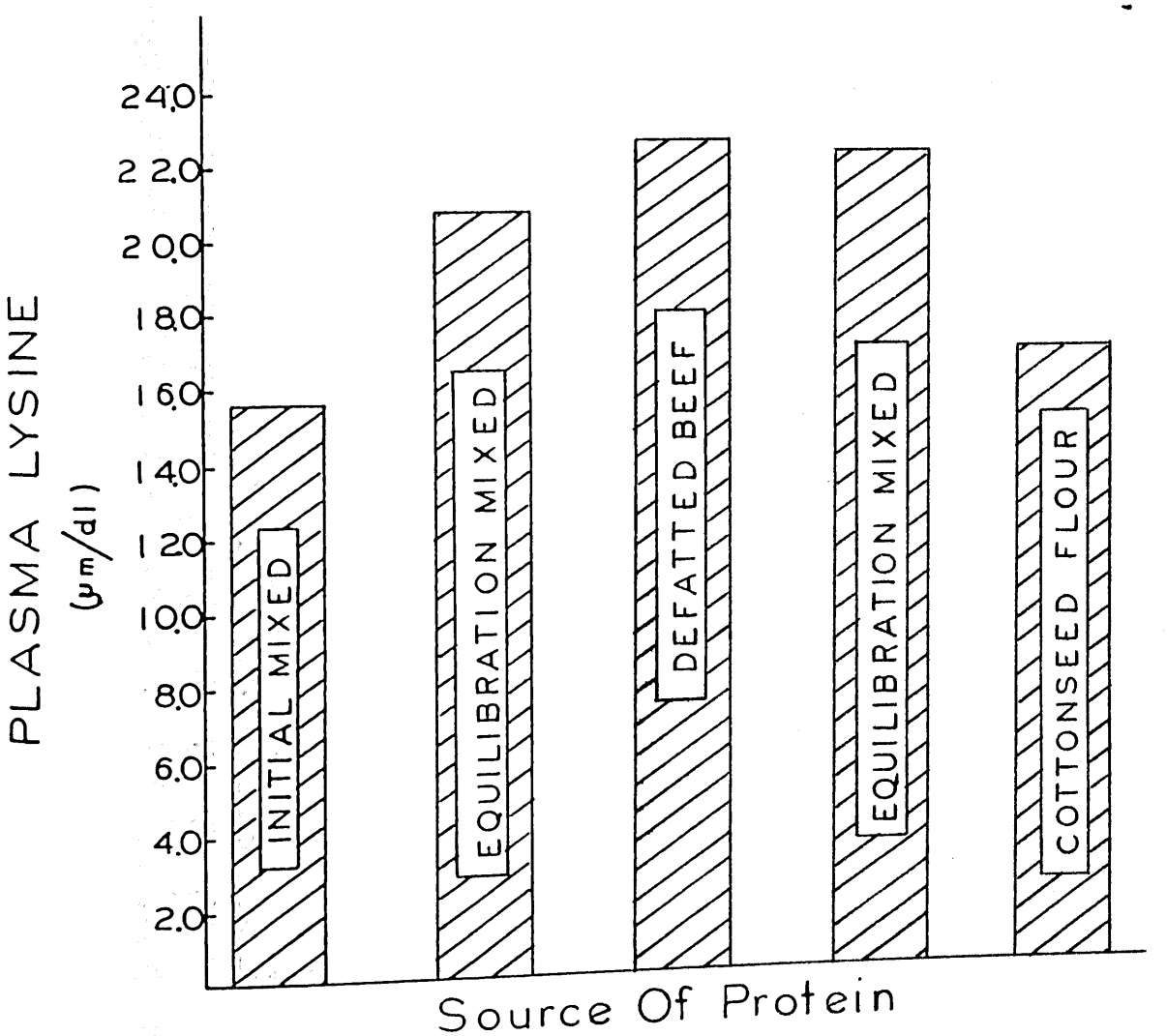

Figure 3. Effect of source of dietary protein on mean concentration of lysine in the plasma of elderly women.

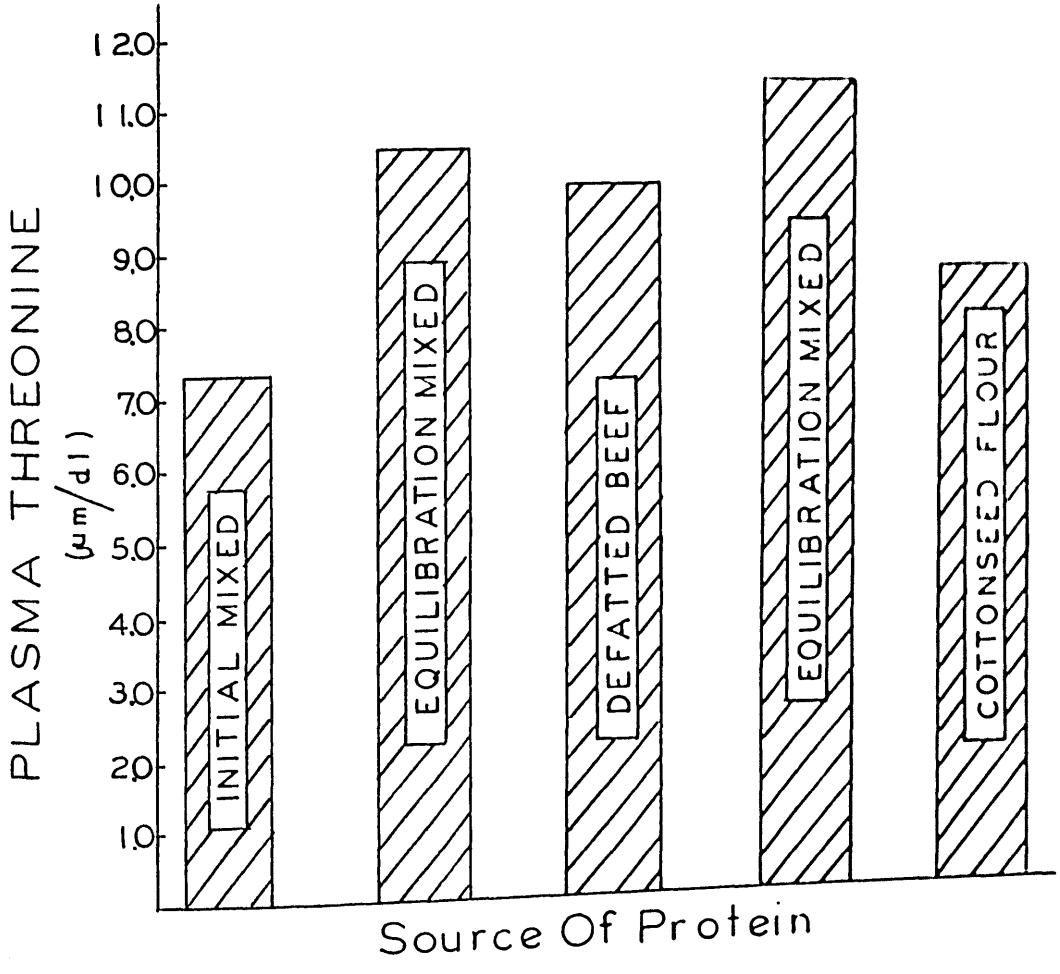

Figure 4. Effect of source of dietary protein on mean concentration of threonine in the plasma of elderly women.

During the cottonseed flour diet, the values were significantly lower (P 0.05) than during the other three dietary periods, but not statistically different from the initial value. Lysine and threonine have been identified as limiting amino acids in cottonseed flour (76, 77), and may have been a contributing factor in the above observation. The low lysine plasma levels found in this investigation are contradictory to work by Young and co-workers (75) who found that despite a zero dietary intake of lysine for periods of seven to twelve days, plasma lysine remained constant. Although plasma lysine in this study was significantly lower during the cottonseed flour diet (16.50 um/dl), it was not abnormally low when compared to 12.37 um/dl reported by Ackermann and Kheim (35) nor 17.7 um/dl reported by Galante and co-workers (33). The value is low, however, in comparison to 20.60 um/dl reported by Ashby (49) for college-age women consuming glandless cottonseed flour as 90% of their protein source. Although the absolute values were higher in Ashby's study, the lowering effect of cottonseed diet study was the same.

The average plasma value for threonine during the cottonseed flour dietary period (8.76 um/dl) was much lower than those reported by Ackermann and Kheim (35) and McCarver (38), 14.55 um/dl and 12.90 um/dl, respectively. The lowering of plasma threonine values found in the present investigation agrees with the findings of

Swenseid (25). Swenseid found that when an amino acid diet is devoid of threonine, its plasma levels will decrease.

Ornithine was significantly higher (P 0.05) during the cottonseed flour diet as compared to the equilibration mixed protein and the defatted beef diets. This finding indicates an effect on the urea metabolic pathway.

Plasma Amino Acid Groupings and Ratios

Plasma amino acids were empirically grouped according to structure and function (Tables VII - XII) to determine if there might have been effects on transport, utilization, or some other metabolic function(s) not obvious when considering individual amino acids. There is competitive inhibition of intestinal absorption between amino acids, and common transport mechanisms between groups of amino acids for tubular reabsorption in the kidney. These could affect plasma amino acid levels if dietary intake of these individual amino acids is disproportionate.

The grouping of the plasma amino acids revealed a similar pattern to that of the individual amino acids. The values for the defatted beef dietary period tended to be the highest; the two equilibration (mixed) values, which were very similar, had intermediate values; and cottonseed flour had the lowest values. The exception to this was the urea cycle intermediates grouping in which the values were higher on the average for the cottonseed flour dietary period. It is interesting to note that arginine, the

TABLE VII

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA UREA CYCLE INTERMEDIATES (um/dl)

Grouping of Amino Acids	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Arginine	8.86± 5.13[1]	12.38± 3.81	11.77± 3.46	11.87± 2.06	13.21± 5.28
Ornithine	7.32± 8.56	6.84± 1.66	6.47± 1.80	6.46± 1.06	8.35± 2.66
Citrulline	2.29± 1.34	4.75± 1.35	4.47± 1.22	4.86± 1.08	4.40± 1.15

[1]Standard deviation.

TABLE VIII

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA BRANCHED-CHAIN AMINO ACIDS (um/dl)

Grouping of Amino Acids	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Isoleucine	2.89± 1.72[1]	4.30± 0.95	9.02±13.09	4.39± 0.93	4.48± 1.63
Leucine	5.98± 3.45	9.19± 2.24	8.91± 2.44	8.90± 1.47	8.56± 2.63
Valine	10.79± 6.18	16.61± 5.25	16.26± 5.83	16.79± 3.87	16.60± 8.40

[1]Standard deviation.

TABLE IX

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA AROMATIC AMINO ACIDS (um/dl)

Grouping of Amino Acids	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Tyrosine	3.55± 2.01[1]	5.64± 1.23	5.05± 1.78	6.01± 1.31	5.46± 2.01
Phenylalanine	2.82± 1.61	4.49± 0.65	4.28± 1.20	4.63± 0.65	3.79± 1.13

[1]Standard deviation.

TABLE X

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA BASIC AMINO ACIDS (um/dl)

Grouping of Amino Acids	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Lysine	15.65± 9.14[1]	20.66± 8.15	22.54± 5.92	22.08± 6.25	16.50± 6.13
Histidine	4.36± 2.44	6.38± 1.24	6.11± 2.13	5.74± 1.01	5.74± 4.36
Arginine	8.86± 5.13	12.38± 3.81	11.77± 3.46	11.87± 2.06	13.21± 5.28

[1]Standard deviation.

TABLE XI

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA HYDROXYLATED AMINO ACIDS (um/dl)

Grouping of Amino Acids	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Serine	8.30± 5.22[1]	10.77± 2.85	9.77± 3.59	10.71± 2.25	11.13± 2.55
Threonine	7.32± 4.58	10.59± 4.58	10.01± 2.91	11.47± 2.64	8.76± 3.81

[1]Standard deviation.

TABLE XII

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA NEUTRAL AMINO ACIDS (um/dl)

Grouping of Amino Acids	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Glycine	23.13±14.17[1]	30.55±11.64	29.80±14.29	31.37±14.02	29.53± 9.89
Alanine	26.44±16.87	38.21±15.07	33.47±12.89	37.78±11.60	34.42±16.09
Proline	16.99±15.71	19.60±12.32	12.07± 5.72	15.29±11.23	13.50±10.62

[1]Standard deviation.

immediate precursor of urea, and ornithine the product of urea formation from arginine were both present in higher amounts during the cottonseed flour period; ornithine was significantly higher (P 0.05). While both the immediate precursor and product of urea formation increased, citrulline, which is formed directly from ornithine was lower. Arginine is present in cottonseed flour at twice the amount present in defatted beef. The cleaving of urea from arginine leaving ornithine is an irreversible reaction (50). This could have contributed to the significant build-up of ornithine in the plasma. Due to the fact that different amino acids were detectable and others missing from the urine, analysis of urine by grouping was not considered.

Another method for studying plasma amino acids is the use of a nonessential (NE) to essential (E) amino acid ratio. The formula shown below has been utilized by Whitehead and Dean (41):

$$NE:E = \frac{\text{glycine+serine+glutamine+taurine}}{\text{isoleucine+leucine+valine+methionine}}$$

Whitehead and Dean found that in children with kwashiorkor, this ratio was higher than in normal children. Sneed found that the ratio increased with decreasing protein intake when the protein was supplied by cottonseed flour. The results here revealed no significant differences between the plasma NE:E ratio in the experimental diets. The values for plasma ratios are given in Table XIII.

TABLE XIII

INFLUENCE OF SOURCE OF DIETARY PROTEIN ON FASTING PLASMA NONESSENTIAL:ESSENTIAL AMINO ACID RATIOS[1]
(um/dl)

Amino Acid	Dietary Source of Protein				
	Initial (Mixed)	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Glycine	23.13±14.17[2]	30.55±11.64	29.80±14.29	31.37±14.02	29.53± 9.89
Serine	8.30± 5.22	10.77± 2.85	9.77± 3.59	10.71± 2.25	11.13± 2.55
Glutamine	18.54±16.80	53.84± 8.16	40.10± 7.83	51.91± 7.25	44.27± 8.04
Taurine	4.20± 2.67	5.44± 1.37	5.64± 1.39	5.38± 1.63	5.20± 2.18
Isoleucine	2.89± 1.72	4.30± 0.95	9.02±13.09	4.39± 0.93	4.48± 1.63
Leucine	5.98± 3.45	9.19± 2.24	8.91± 2.44	8.90± 1.47	8.56± 2.63
Valine	10.79± 6.18	16.61± 5.25	16.26± 5.83	16.79± 3.87	16.60± 8.40
Methionine	1.37± 0.86	1.98± 0.49	2.12± 0.59	1.98± 0.37	2.14± 0.71
Ratio	2.22± 1.27	3.05± 1.04	2.71± 0.77	2.87± 0.61	2.91± 0.80

[1]Nonessential:Essential Amino Acid Ratio =

$$\frac{\text{glycine+serine+glutamate+taurine}}{\text{isoleucine+leucine+valine+methionine}}$$

[2]Standard deviation.

Free Amino Acids in Urine

Mean excretory values for the 13 free amino acids found in the urine are presented in Table XIV. Of the 13 amino acids, four showed significant differences between the dietary periods. Taurine, methionine, cystathionine, and 3-methylhistidine were excreted in significantly greater amounts (Figures 5-8) during the defatted beef dietary period as compared to the other dietary period (P 0.05). Taurine and methionine were also found to be excreted in significantly lesser amounts (P 0.05) during the cottonseed flour dietary period as compared with the others. These findings are similar to those of Ashby (49). Of the four amino acids for which significant differences were found, taurine, cystathionine, and methionine are sulphur-containing compounds and share a common metabolic pathway (Figure 9). Urinary taurine can serve as a crude index of taurine status (77). Taurine plays an important role in regulating cholesterol metabolism via the glycine/taurine ratio in bile (77).

Approximately a six-fold difference in urinary excretion of methionine occurred between the cottonseed flour and defatted beef dietary periods. Table XV shows the three-fold difference in dietary methionine from the two protein sources. However, the plasma methionine and taurine levels remained virtually unchanged throughout the study.

TABLE XIV

FREE AMINO ACIDS FOUND IN URINE OF ELDERLY SUBJECTS[1] (um/24 hrs)

Amino Acid	Equilibration (Mixed)	Defatted Beef	Equilibration (Mixed)	Cottonseed Flour
Serine	56.31± 39.61[2]	65.31± 57.28	77.36± 62.32	72.45± 60.18
Taurine	304.96±195.91	601.51±265.00[3]	365.09±218.65	185.84±142.68
Phosphoethanolamine	353.32±112.97	423.20±169.94	378.00±125.17	351.64± 99.46
Phosphoserine	35.36± 12.97	35.33± 12.08	36.05± 9.97	39.16± 9.51
Methionine	10.36± 4.84	30.67± 12.99[3]	11.28± 3.91	5.53± 3.29
Histidine	134.92±106.92	147.71± 89.91	152.02± 58.73	181.98± 70.27
Alanine	108.54± 45.98	123.28± 28.07	123.68± 28.07	116.81± 38.14
Glycine	833.44±498.61	735.88±427.41	654.63±449.23	712.62±449.23
3-Methylhistidine	110.82± 72.27	191.32± 93.86[3]	108.78± 36.16	87.61± 21.03
Cysteine	51.95± 16.15	66.60± 20.29	63.50± 17.06	63.72± 20.63
Leucine	14.05± 10.16	11.92± 8.90	14.14± 12.04	13.60± 8.61
Threonine	15.42± 20.51	20.86± 23.10	23.63± 25.66	24.79± 21.43
Cystathionine	9.82± 6.24	15.03± 4.07[3]	10.16± 5.98	11.99± 5.30

[1]Values based on average 24 hour excretion from a 5-day pool.

[2]Standard deviation.

[3]Significantly higher than all other values (P .05).

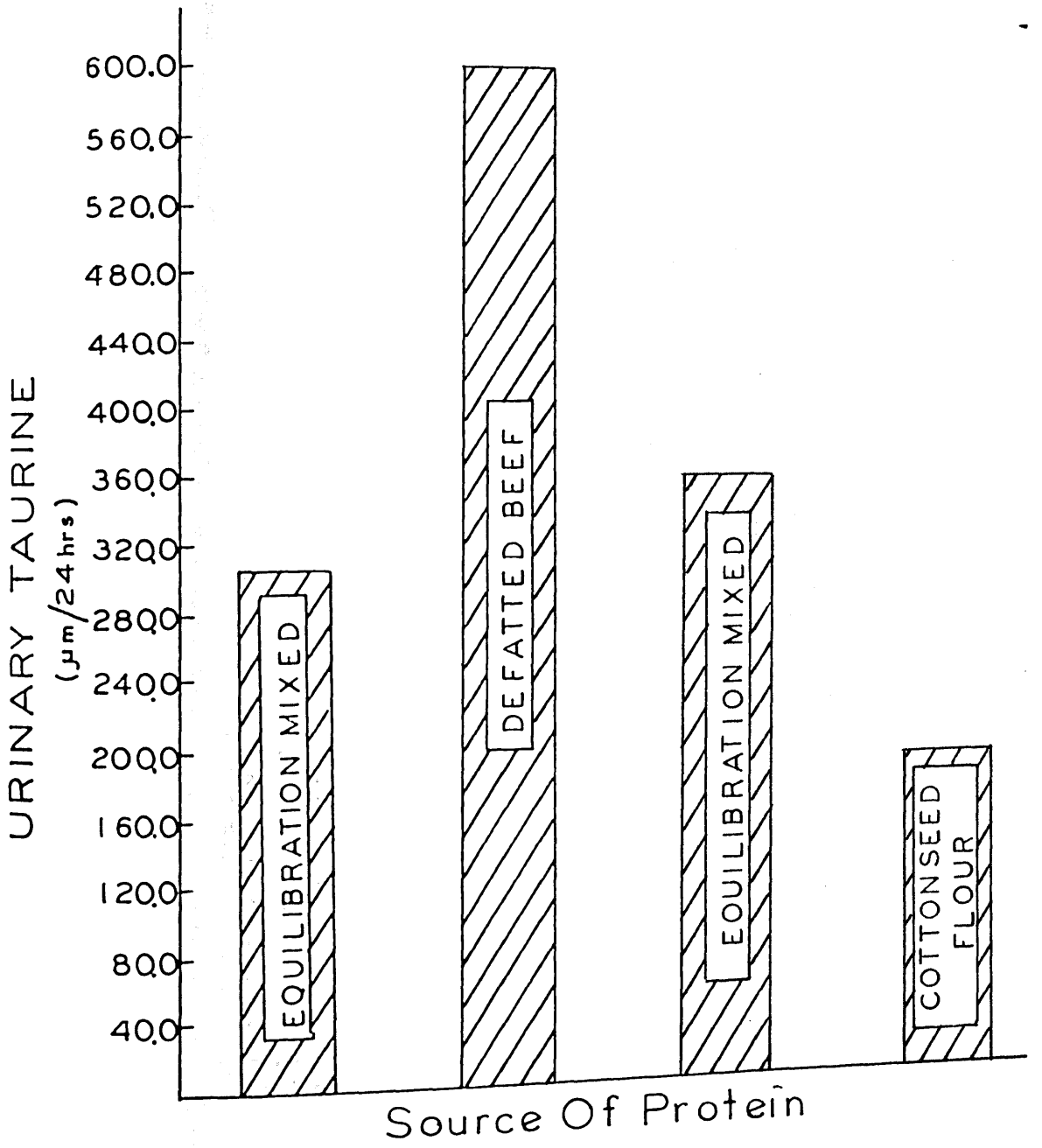

Figure 5. Effect of source of dietary protein on 24-hour urinary excretion of taurine in elderly women.

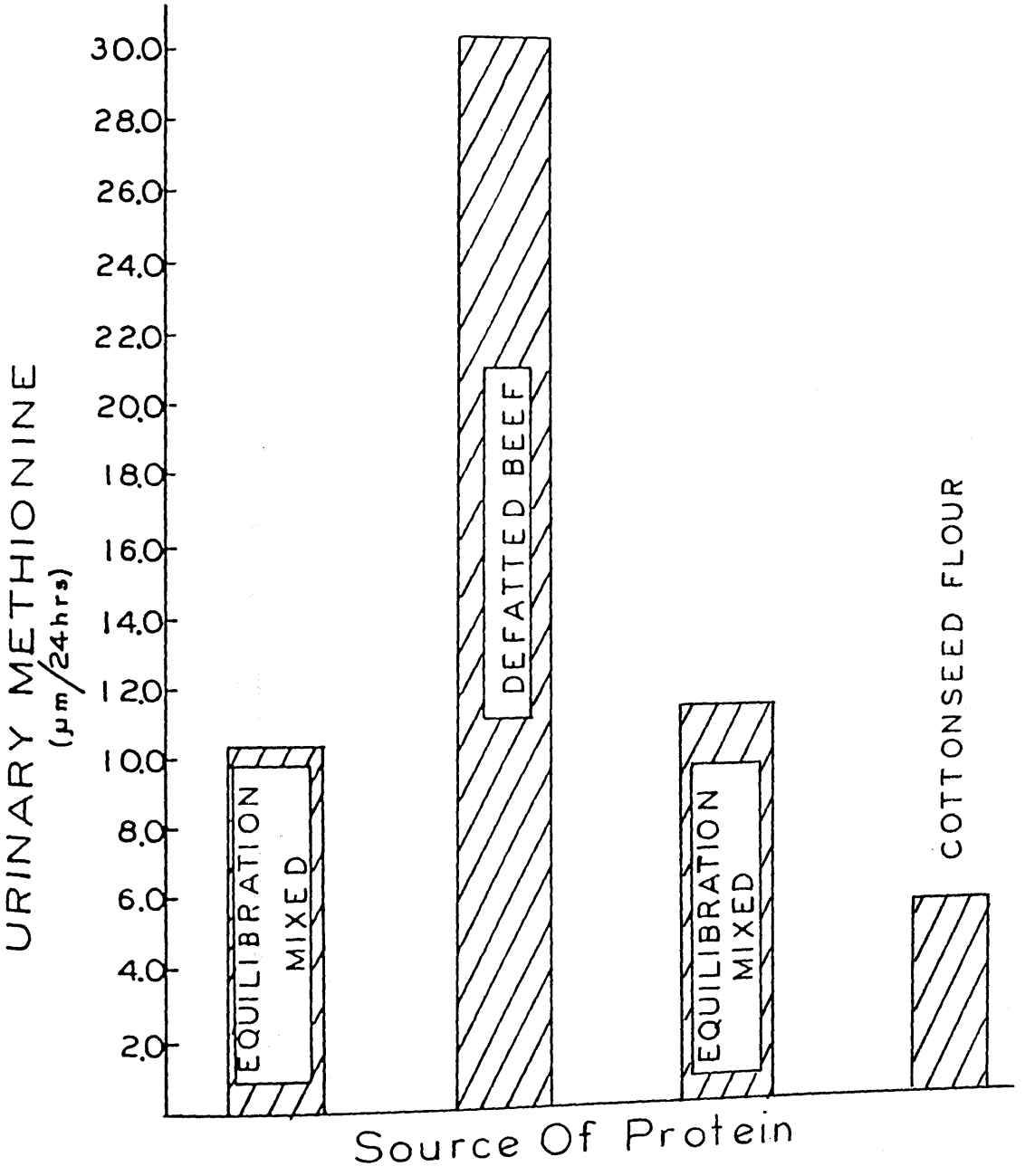

Figure 6. Effect of source of dietary protein on 24-hour urinary excretion of methionine in elderly women.

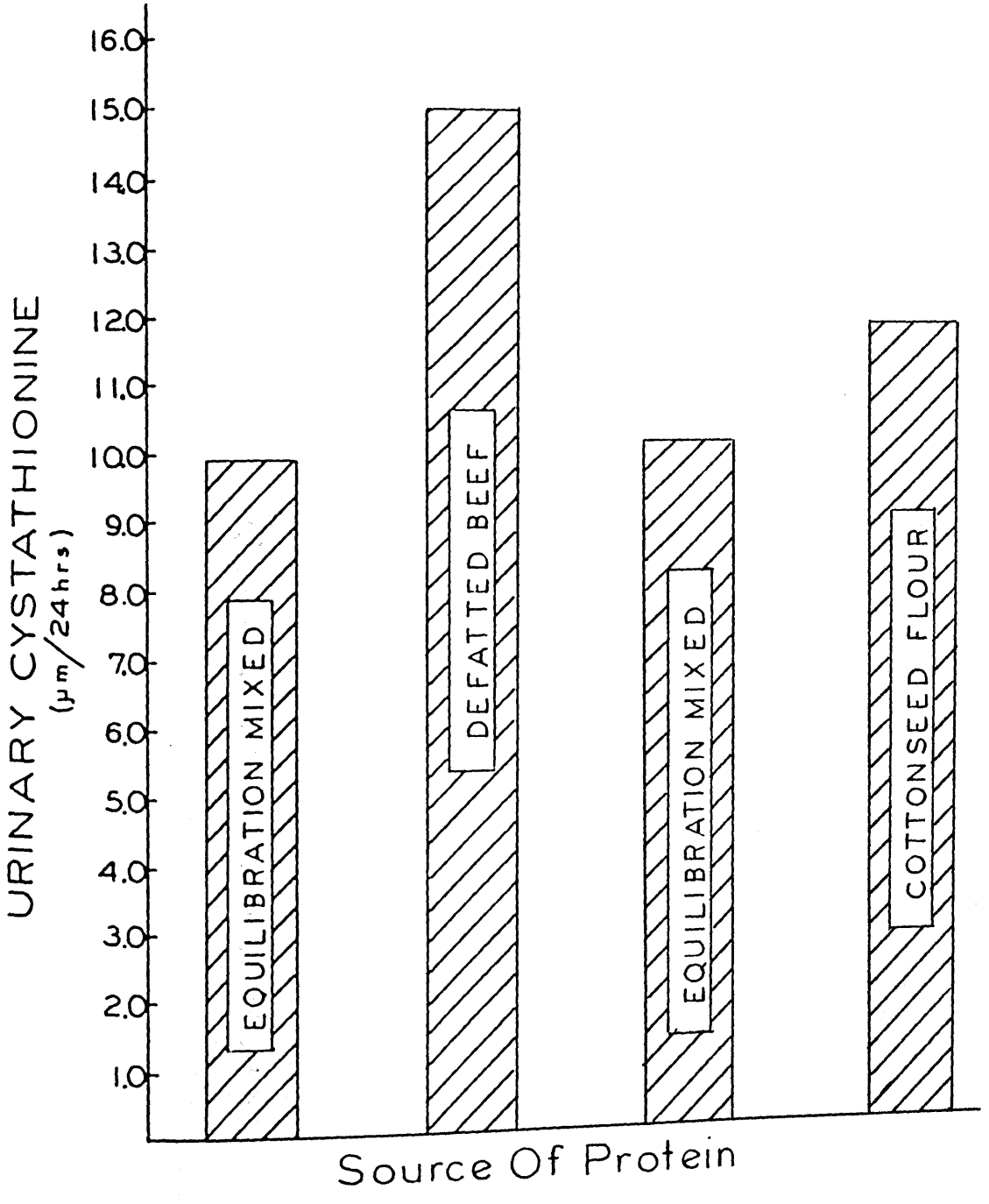

Figure 7. Effect of source of dietary protein on 24-hour urinary excretion of cystathionine in elderly women.

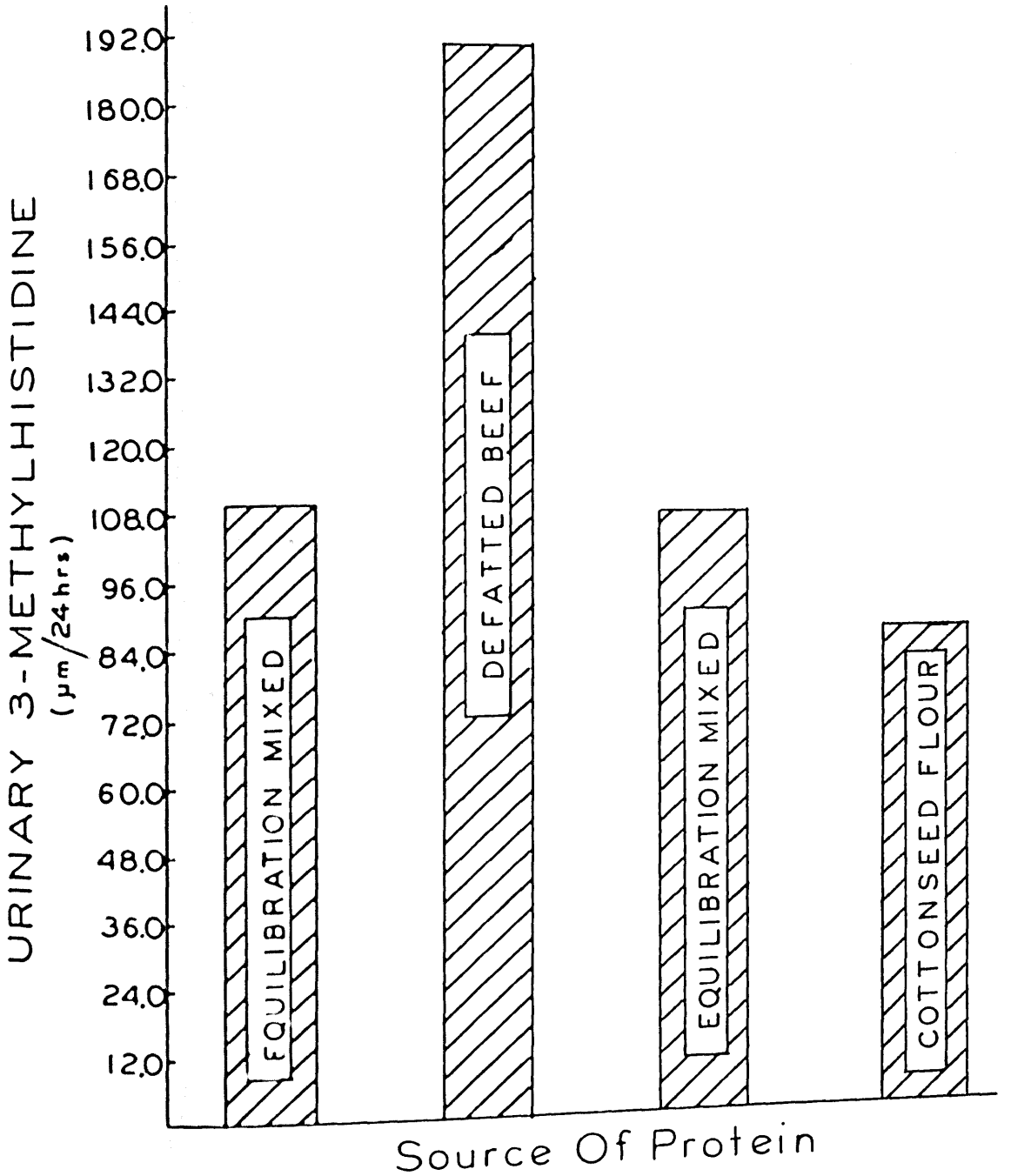

Figure 8. Effect of source of dietary protein on 24-hour urinary excretion of 3-methylhistidine in elderly women.

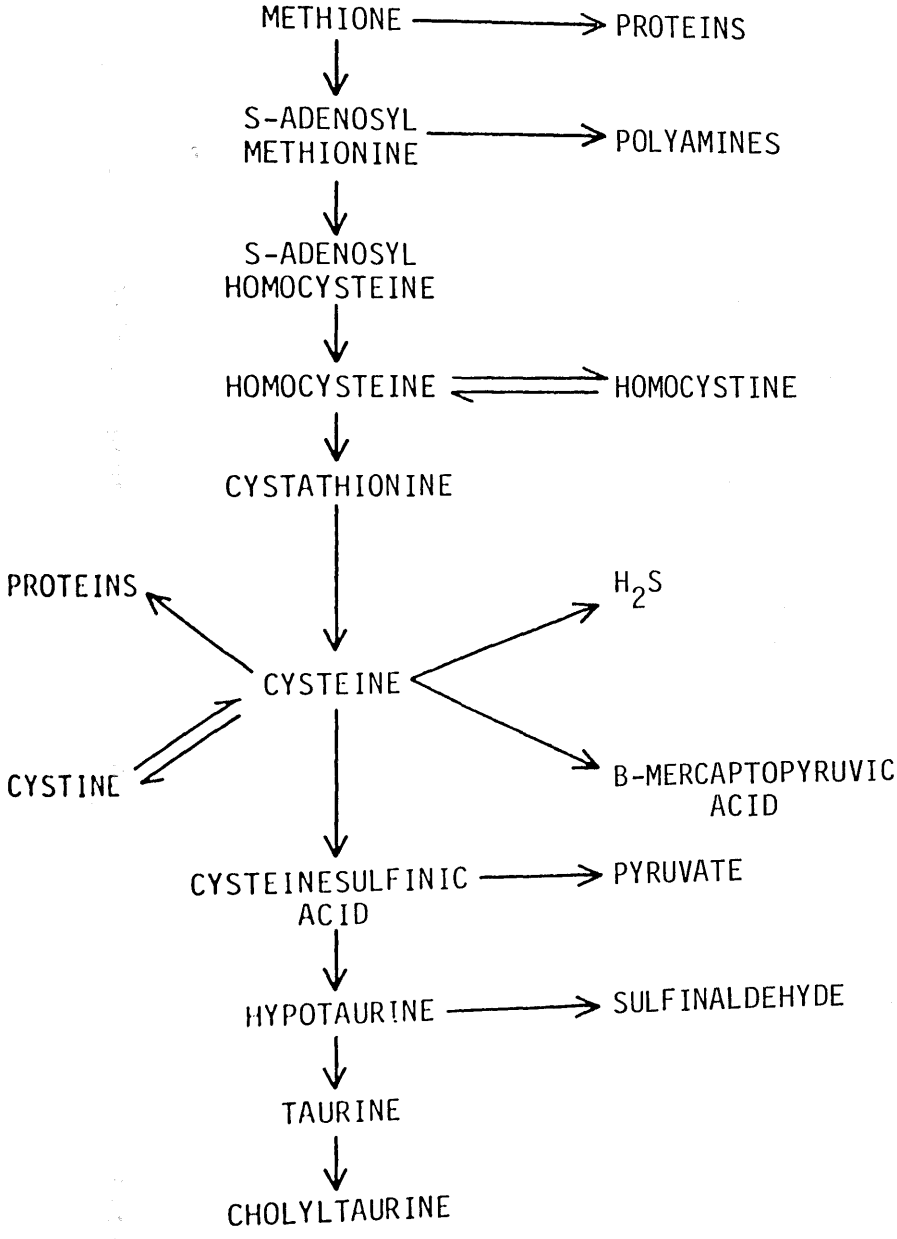

Figure 9. Metabolic pathway shared by methionine, cystathionine, and taurine (74).

TABLE XV

COMPARISON OF THE PLASMA AND URINARY AMINO ACID VALUES RELATIVE TO DIETARY PROTEIN SOURCE

Amino Acid	Product	Product Composition (g/100g protein)	Mean Plasma (um/dl)	Mean Urinary (um/24 hrs.)
Histidine	Defatted Beef	3.11	6.11	149.71
	Cottonseed Flour	2.76	5.74	181.98
Isoleucine	Defatted Beef	4.71	9.02	N.A.
	Cottonseed Flour	2.74	4.48	N.A.
Leucine	Defatted Beef	7.96	8.91	11.92
	Cottonseed Flour	5.08	8.55	13.60
Lysine	Defatted Beef	8.38	22.66[1]	N.A.
	Cottonseed Flour	4.07	16.50	N.A.
Methionine	Defatted Beef	6.23	2.12	30.67[1]
	Cottonseed Flour	2.11	2.14	5.54
Phenylalanine	Defatted Beef	4.12	4.28	N.A.
	Cottonseed Flour	5.19	3.97	N.A.
Threonine	Defatted Beef	4.78	10.01[1]	65.31
	Cottonseed Flour	2.88	8.76	72.45
Valine	Defatted Beef	4.87	16.26	N.A.
	Cottonseed Flour	4.21	16.60	N.A.

[1]Significantly greater (P .05) than cottonseed flour value.

TABLE XV-Continued

Amino Acid	Product	Product Composition (g/100g protein)	Mean Plasma (um/dl)	Mean Urinary (um/24 hrs.)
Alanine	Defatted Beef	6.26	33.47	123.28
	Cottonseed Flour	6.40	34.42	116.81
Arginine	Defatted Beef	6.31	11.77	N.A.
	Cottonseed Flour	11.42	13.21	N.A.
Glycine	Defatted Beef	5.94	29.80	735.88
	Cottonseed Flour	5.90	29.53	712.62
Proline	Defatted Beef	4.74	12.07	N.A.
	Cottonseed Flour	3.83	13.50	N.A.
Serine	Defatted Beef	3.99	3.59	65.31
	Cottonseed Flour	4.02	2.55	72.45
Tyrosine	Defatted Beef	3.42	5.05	N.A.
	Cottonseed Flour	2.88	5.46	N.A.

The excretion of 3-methylhistidine has been related to the nutritive quality of a dietary protein in rats (72). The better the protein quality, as determined by net protein quality, as determined by net protein utilization, the higher is the total urinary 3-methylhistidine excretion. Marliss and co-workers (73) examined the effects of dietary 3-methylhistidine on its urinary excretion in humans. The lowest values occurred without meat as a protein source, intermediate values occurred with mixed proteins and higher values were found with beef as the sole protein source. The same results occurred in the present investigation. It is impossible to ascertain if the increased 3-methylhistidine excretion during the defatted beef dietary period was due to the higher protein quality or the presence of 3-methylhistidine in the beef muscle causing a quantitative increase in its excretion.

Relationship Between Plasma and Urinary Free Amino Acids

Simple correlations were calculated using the means of the amino acids that were measured in both plasma and urine and for both experimental periods. Only one correlation was significant, and that was the negative correlation between plasma and urinary serine during the defatted beef dietary period, (P 0.05). Six of the nine amino acids were found to have negative correlations during the defatted beef dietary period, although only serine was significant.

No significant correlations between plasma and urinary values were found during the cottonseed dietary period although seven of the nine were positive. A comparison of the plasma and urinary amino acid composition with the dietary protein source can be seen in Table XV.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present investigation examined the effects of different dietary proteins on the fasting plasma and urinary free amino acids in elderly women. The dietary proteins consisted of a mixed protein, defatted beef, or glandless cottonseed flour. The diets were administered in a crossover design as discussed in the text.

Significant decreases ($P < 0.05$) in fasting plasma lysine and threonine occurred with the cottonseed flour diet as compared to the mixed protein or defatted beef. In studies by Elias and Bressani (76), the limiting amino acids of deglanded cottonseed protein were found to be lysine, threonine, and methionine, in that order. Although the plasma lysine was lower, the plasma values never fell below the normal range reported in the literature, indicating adequate plasma lysine status of the subjects during the cottonseed protein dietary period.

Thirteen of the initial plasma amino acid values were lower than those in the literature for elderly women. Ten of these were significantly lower than the values for the remainder of the study. Values for valine, threonine, and leucine remained lower than literature values throughout the investigation. The finding that the initial concentration of most plasma acids were lower than

normal, and that these amino acids increased upon initiation of the study, indicates that the subjects' previous protein intake was well below the 0.8 g per kilogram provided during the study. The increase in plasma amino acids that occurred during the cottonseed protein dietary period, provides evidence that the cottonseed protein improved amino acid status over the subject's status prior to the study.

The urea cycle intermediate grouping of plasma amino acids revealed an increase in arginine and ornithine during the cottonseed flour dietary period. Ornithine was significantly higher (P 0.05). For future study, quantitation of blood urea nitrogen might provide a more complete picture of what is occurring in the urea cycle. The nonessential to essential amino acid ratios were not significantly different between dietary periods. This index has been used previously as an indicator of inadequate protein intake (39, 41), thus suggesting that protein intake was adequate in this investigation during the cottonseed protein dietary period.

Although the plasma levels of methionine remained virtually the same throughout the study, urinary excretion of methionine and its metabolites, cystathionine, and taurine were significantly higher during the defatted beef dietary period.

Significant differences were found in the urinary excretion of taurine, methionine, cystathionine, and 3-methylhistidine between dietary periods. Taurine, methionine, and cystathionine are

sulphur-containing compounds. The greater dietary methionine in the defatted beef, providing more precursor for cystathionine and taurine, as well as methionine excretion could explain the greater urinary excretion of the sulphur-containing compounds.

Taurine excretion was greatest during the defatted beef dietary period, intermediate during the mixed protein and lowest during the cottonseed protein dietary period. Taurine, as a amino compound is poorly reabsorbed by the kidney and readily excreted. Muscle is the largest reservoir of taurine, whereas it is essentially absent in plants. One explanation for the higher urinary taurine during the defatted beef dietary period and the lower excretion during the cottonseed protein is the presence of taurine in the beef and the absence of it in the cottonseed.

Histidine is methylated in peptide linkage within muscle protein, and is quantitatively excreted when the protein is catabolized (73). Omstedt and co-workers (72) demonstrated that a relationship exists between the nutritive quality of a dietary protein and the urinary excretion of 3-methylhistidine in the rat. Marliss (73) has added that if 3-methylhistidine is ingested with muscle protein, as would be expected, it would be quantitatively excreted. Thus, urinary excretion could be a direct reflection of dietary intake of the compound rather than nutritive quality of the protein. In this investigation, a significant increase in urinary excretion of 3-methylhistidine occurred during the defatted beef

dietary period. Excretion was intermediate with mixed protein, and lowest with glandless cottonseed flour. For future research analysis of the defatted beef for 3-methylhistidine and taurine, and analysis of the cottonseed flour for taurine would provide easier interpretation of a cause and effect relationship between the dietary protein and the urinary amino acid excretion.

Urinary leucine and alanine have been shown to decrease during protein deprivation (44). No such alteration was observed in this study, again indicating that protein was adequate during the cottonseed protein dietary period.

In conclusion, this investigation into the comparative effects of beef, cottonseed, and mixed protein diets on the amino acid status of healthy, elderly women found cottonseed protein to be comparable to that of defatted beef and mixed protein. Lysine and threonine, as limiting amino acids in cottonseed, were lower in the plasma during that dietary period. However, plasma lysine levels never fell below the normal range even though cottonseed provided 90% of the protein in the diet. Plasma threonine as well as valine and leucine remained below literature values throughout the investigation.

Based on this investigation, if cottonseed protein were one of several protein sources, rather than as 90% of protein, minimal changes in plasma threonine and lysine would be anticipated.

APPENDICES

APPENDIX A

FOUR-DAY CYCLE MENU

EQUILIBRATION DIET

Day	Breakfast	Lunch	Supper
I	Scrambled Egg Orange Juice Toast Margarine/Jelly	Baked Flounder Mashed Potato Carrots/Margarine Lettuce Salad French Dressing	Chicken Salad Tomato Lettuce/Apple Sugar Wafers Milk
II	Grapefruit Sections Scrambled Egg Toast Margarine/Jelly Milk	Rump Roast Mashed Potatoes Beets/Margarine Lettuce Salad Thousand Island Dressing Sherbet	Fruit Medley Cottage Cheese Slice Am. Cheese Crackers Sugar Wafers
III	Orange Juice Raisin Bran Milk Toast Margarine/Jelly	Chicken Breast Rice/Margarine Green Beans Apple	Tuna Noodle Casserole Relish Plate Ice Cream
IV	Grapefruit Section Cornflakes Milk Toast Margarine/Jelly	Ham Baked Potato Broccoli Margarine Canned Pears	Grilled Cheese Sandwich Vegetable Beef Soup Carrot and Celery Sticks Sugar Wafers

APPENDIX B

FIVE-DAY CYCLE MENU

EXPERIMENTAL DIET

Day	Breakfast	Lunch	Supper
I	Apple Crisp	Brown Ground Tagliatelle Carrots/ Margarine Apple	Chili Rigatini/Margarine Cole Slaw Low PRO Gelatin
II	Kugal	Chow Mein Rice/Margarine Canned Pineapple Low PRO Gelatin	Tomato Sauce Spaghetti/ Margarine Lettuce Italian Dressing
III	Apple Crisp	Sweet 'N Sour Rice Margarine Carrots Canned Peaches	Gumbo Rigatini/Margarine Canned Pears
IV	Kugal	Soup Green Beans Margarine Baked Apple	Zucchini Skillet Dish Carrots/Margarine Canned Pears
V	Hot Spiced Fruit	Cabbage Apple Skillet Noodles/ Margarine Canned Peaches	Goulash Lettuce Italian Dressing Canned Pears

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