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THE INFLUENCE OF SOYBEAN COMPARED WITH SOYBEAN-EGG
DIETS ON PROTEIN-CALORIE STATUS AND
PROTEIN METABOLISM
IN HEALTHY MEN

PREEYA LEELAHAGUL

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Preeya Leelahagul

ได้ศึกษาถึงอิทธิพลของอาหารถั่วเหลืองเปรียบเทียบกับอาหารถั่วเหลืองและไข่
ต่อภาวะโภชนาการด้านโปรตีนและพลังงานและเมตาบอลิซึมของโปรตีนในผู้ชายปกติ ผู้ได้
รับการศึกษาคือชายปกติ 7 คน อายุ 30-35 ปี ที่หอวิจัยทางคลินิก คณะแพทยศาสตร์
โรงพยาบาลรามาธิบดี เป็นระยะเวลา 25 วัน โดยแบ่งระยะเวลาการศึกษาดังนี้
วันที่ 1 และ 15 ให้รับประทานอาหารไม่มีโปรตีน (PF) วันที่ 2-11 เป็นอาหาร
มังสวิรัต (VD) วันที่ 12-14 เป็นอาหารปกติ วันที่ 16-25 เป็นอาหารมังสวิรัตเสริม
ไข่ (OV) พลังงานที่ผู้ได้รับการศึกษาแต่ละคนได้รับตลอดการศึกษาเท่ากับปริมาณพลังงาน
โดยเฉลี่ยที่คำนวณได้จากช่วงก่อนการศึกษา 14 วัน (BL) ของแต่ละคนบวกกับค่าเบี่ยง-
เบนมาตรฐาน (LSD) ในช่วง VD และ OV การกระจายของพลังงานของอาหาร
ที่ได้รับเป็นดังนี้ ร้อยละ 15 จากโปรตีน ร้อยละ 30 จากไขมัน และร้อยละ 55 จาก
คาร์โบไฮเดรต แหล่งของโปรตีนในช่วง VD มาจากถั่วเหลืองร้อยละ 90 และข้าวร้อยละ

10 ส่วนในช่วง OV มาจากถั่วเหลืองร้อยละ 72 ใช้ร้อยละ 18 และข้าวร้อยละ 10 ผลการศึกษาพบว่าเมื่อให้ปริมาณพลังงานและโปรตีนเท่ากันการทดแทนโปรตีนจากถั่วเหลืองด้วยโปรตีนจากใช้ร้อยละ 18 นี้ ไม่เห็นผลแตกต่างกันต่อคูลบวกไนโตรเจน apparent digestibility, apparent biological value, net protein utilization และไม่มีการเปลี่ยนแปลงของ body muscle mass, upper arm muscle circumference, และ bone-free arm muscle area ระดับของ serum total protein และ albumin อยู่ในระดับปกติตลอดการศึกษา ระดับ serum transferrin ตลอดการศึกษามีแนวโน้มต่ำกว่าช่วง BL ทั้งนี้อาจเป็นเพราะปริมาณพลังงานที่ได้รับไม่เพียงพอแต่ได้รับโปรตีนเพียงพอกับความต้องการ ส่วนระดับ serum retinol-binding protein ลดลงตลอดการศึกษาเพราะวิตามินเอที่ได้รับอาจต่ำกว่าความต้องการเนื่องจากได้รับอยู่ในสภาพของแคโรทีนเท่านั้นในช่วงของ VD และส่วนใหญ่ในช่วงของ OV การศึกษาของเราพบว่าระดับ urinary creatinine ไม่คงที่ทั้งในคนคนเดียวกันและในแต่ละวัน นอกจากนี้การศึกษพบว่าระดับ creatinine ที่ได้จากอาหารมีผลต่อ ระดับ urinary creatinine มากกว่าระดับโปรตีน ค่า creatinine coefficient ในกลุ่มผู้ได้รับการศึกษาโดยเฉลี่ย 15 มก./กก. ซึ่งต่ำกว่าค่าของชาวอเมริกัน แสดงถึงว่ากลุ่มผู้ได้รับการศึกษาของเรามี muscle mass น้อยกว่าและบริโภคเนื้อสัตว์น้อยกว่า ค่า creatinine clearance มีการเปลี่ยนแปลงตลอดการศึกษา ทั้งนี้เนื่องจากระดับ urinary creatinine ไม่คงที่ ระดับ urinary uric acid มีความสัมพันธ์ทางบวกกับระดับโปรตีนที่ได้จากอาหาร และระดับ nucleic acid จากอาหารมีผลต่อการเปลี่ยนแปลงของ urinary uric acid แต่ปริมาณโปรตีนจากถั่วเหลืองร้อยละ 13.5 ของพลังงานทั้งหมดไม่ทำให้เกิดภาวะ hyperuricemia และ uricosuria การศึกษานี้ได้ชี้ให้เห็นว่าสามารถนำถั่วเหลืองมาใช้เป็นแหล่งให้โปรตีนเพื่อเสริมคุณค่าทางโภชนาการของอาหารที่มีข้าวเป็นส่วนประกอบหลัก

Thesis Title The influence of soybean compared with
 soybean-egg diets on protein-calorie status
 and protein metabolism in healthy men

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ABSTRACT

This study was carried out to investigate the influence of soybean compared with soybean-egg diets on protein-calorie status and protein metabolism. Seven healthy men, aged 30-35 yr, participated in a 25-day metabolic study. They received protein-free diet (PF) for days 1 and 15, vegetarian diet (VD) for days 2-11, regular diet (RD) for days 12-14, and ovovegetarian diet (OV) for days 16-25. Energy intake for each subject receiving PF, VD, or OV was held constant according to his energy intake during the baseline (BL) period plus 1 SD whereas RD was served ad libitum. Energy distributions of VD and OV were 15, 30, and 55% derived from protein, fat, and carbohydrate, respectively. The protein sources of VD were soybean (90%) and rice (10%) whereas those of OV were

soybean (72%), egg (18%), and rice (10%). The substituting soybean protein by egg protein while total nitrogen and energy intake being kept constant no further increase in positive nitrogen balance was observed. This is consistent with (a) the similar figures of apparent digestibility, biological value, and net protein utilization of VD and OV, and (b) the anthropometric data which revealed the preservation of the somatic protein status. All of the subjects had serum total protein and albumin levels in the acceptable levels throughout the study. The decrease in their mean serum transferrin levels during the study was most likely due to inadequate energy intake. The decrease in their serum RBP levels was possibly due to low vitamin A intake derived from carotene. The creatinine coefficient in our subjects on the BL diet was 15 mg/kg which was lower than that in American subjects. This reflects lower muscle mass and lower meat protein intake in our subjects. Their creatinine clearance varied considerably because of the variation in urinary creatinine excretion. The influence of dietary protein and nucleic acid intake on urinary uric acid level is also evident in our study by the significant positive correlation between the protein intake and urinary uric acid excretion. The soybean protein intake at 13.5% of total dietary energy did not cause hyperuricemia and uricosuria. Based on the overall results, we have shown the benefits of soybean complementation to rice-based diet.

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CHAPTER I

PROBLEMS AND OBJECTIVES

Statement of the problems

Protein-calorie malnutrition (PCM) is one of the major problems affecting the life and well-being of various populations in developing countries. Their total protein intake is usually lower than those in developed countries. Besides, in the former cereals are their main protein sources whereas those in the latter are meat and dairy products (1). The prevalence of PCM is alarming, ranging from 20-80% of preschool children (2). Supplementation with protein-rich foods have been recognized as the important mode to alleviate the problem of PCM (3).

Inadequate protein-calorie status is also common in rural Thai adults (4). Their protein and energy needs are mainly supplied from a rice-based diet. The bulkiness of this diet makes it difficult to meet their energy requirement (5,6). The nutritional quality of their protein intake is also low because rice is the major dietary source of protein evidenced by low serum albumin, and low serum and urinary carnitine levels suggesting lysine deficiency (5). In addition to carnitine deficiency, essential fatty acid depletion usually coexists in this free-living population (6).

Objectives and specific aims

Based on the aforementioned problems, we are interested in investigating the effect of soybean as the major dietary protein source on protein-calorie status and protein metabolism in adults.

The specific aims of the study are to investigate the influence of soybean compared with soybean-egg diets on protein-calorie status and protein metabolism determined by

1. dietary assessment,
2. anthropometric assessment,
3. nitrogen balance,
4. visceral protein status,
5. creatinine metabolism, and
6. uric acid metabolism

CHAPTER II

MATERIALS AND METHODS

Subjects

Eight healthy men participated in a 14 days of baseline (BL) study and a 25-days of metabolic study. Table 1 shows their age, height, body weight, and occupation.

Diets

During the metabolic study, all of the subjects resided in the Clinical Research Ward, Ramathibodi Hospital, Bangkok, Thailand. They received the following diets: protein-free diet (PF) for days 1 and 15, vegetarian diet (VD) for days 2-11, regular diet (RD) for days 12-14, and ovo-vegetarian diet (OV) for days 16-25. Energy intake for each subject receiving PF, VD, or OV was held constant according to his energy intake during 14 days of the BL period whereas RD was served ad libitum. Energy distributions of VD and OV were 15, 30, and 55% derived from protein, fat, and carbohydrate, respectively. The protein sources of VD were soybean (90%: soybean 22%, texturized protein 25%, and soybean curd cake 43%) and rice (10%) whereas those of OV were soybean (72%: soybean 17%, texturized protein 20%, and soybean curd cake 35%), egg (18%), and rice (10%). Only soybean oil was used for

cooking and contributed 20% of total energy intake. The protein sources of RD were chicken and rice and the main fat source was soybean oil.

Dietary assessment

During the BL period, dietary intake in each subject was assessed by the 24-hr dietary record. Protein, fat, carbohydrate, and total energy intake were then calculated (7,8). Energy intake for each subject receiving PF, VD, or OV was held constant according to his mean energy intake plus 1 SD observed during the 14 days of the BL period.

Table 2 shows menus served during the PF period. Table 3 shows 30 menus served at various days of VD and OV. The protein sources of VD were soybean and rice with the ratio of 9:1 whereas those of OV were soybean, egg, and rice with the ratio of 7.2:1.8:1.0. Soybean was served as whole soybean and soybean products. Table 4 shows 9 menus served at various days of RD period. The protein sources of RD were chicken and rice with varying ratio according to rice consumption. Daily dietary intake in each subject during PF, VD, RD, and OV was recorded and calculated for protein, fat, carbohydrate, and total energy intake.

Daily amino acid and purine intake in each subject on VD and OV were calculated by using published references (9-10).

Basal energy expenditure (BEE) in each subject during BL was estimated by Harris-Benedict equation (11): $BEE = 66 + (13.7 \times W) + (5 \times H) - (6.8 \times A)$ where W=actual weight in kg, H=height in centimeter, and A=age in years. Since all of the subjects had light activities their estimated energy requirements (EER) were calculated as $1.55 \times BEE$ (11).

Anthropometric measurement

The following anthropometric measurement was carried out at day 1 (BL): before receiving protein-free diet), day 12 (at the end of VD), day 15 (at the end of RD), and day 26 (at the end of OV): body weight, triceps, biceps, subscapular and suprailiac skinfold thicknesses (TSF, BSF, SSF, SISF), and mid upper arm, waist, and hip circumferences. Height was measured at day 1.

Percentage of body fat was estimated by the sum of the four skinfold thicknesses described by Durnin and Womersley (12). Other anthropometric parameters were calculated as follows:

2

body mass index (BMI) in kg/m^2 (13) = body weight
in $\text{kg}/(\text{height in meter})^2$

mid upper arm muscle circumference in cm (14) =
upper arm circumference in cm - $3.1416 \times \text{TSF in cm}$

fat-free mass in kg (15) = body weight in kg - body
fat in kg

bone-free arm muscle area in cm^2 (15) = (upper arm muscle circumference, in cm) ^{2} /4 -10

body muscle mass in kg (15) = height in cm (0.0264 + 0.0029 x bone-free arm muscle area, in cm^2)

Collection of blood, urine, and feces

Blood was collected after a 12-hr fast at the following intervals together with the anthropometric measurements: at days 1, 12, 15, and 26 for hematological and biochemical determination.

Twenty-four hr urine samples were collected during the last 3 days of the BL period and the 25 days of metabolic period. Urinary total nitrogen, creatinine and uric acid were analysed from each sample of each subject.

Carminc dye capsules (200 mg/capsule) were given to serve as a fecal marker to indicate the beginning and end of each 5-day collection of VD and OV period. Pooled stool collections of each period were analyzed for total nitrogen.

Statistical analysis

Conventional statistical methods were used for the calculation of mean, standard deviation (SD), standard error of mean (SEM) paired or unpaired Student's t test (two-tailed), and linear regression analysis (16). Subject no.8 was excluded from the analysis because he developed

upper respiratory tract infection and received antibiotic treatment during the study.

Hematological determination

Hemoglobin (Hb), hematocrit (Hct), red blood cell (RBC) count, white blood cell (WBC) count, platelet count, RBC indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined by Hemolog 8 and Hemolog D (Technicon Instruments Corp, Tarrytown). Total lymphocyte count was derived from WBC count $\times$ % lymphocyte/100.

Biochemical determination

Serum total protein was measured by biuret assay (17), albumin by dye-binding technique (18), transferrin and retinol-binding protein (RBP) by the method modified from Mancini et al (19).

Plasma glucose was determined by glucose oxidase method (20) and serum urea nitrogen by the Fearon reaction (20). Serum and urinary creatinine were determined by Jaffe reaction (20) and serum and urinary uric acid by uricase method (20).

Urinary and fecal total nitrogen were determined by Kjeldahl method (21), using Kjeltac system (Tecator, Sweden). Nitrogen balance was calculated from nitrogen

intake-urinary nitrogen-fecal nitrogen-unmeasured nitrogen including 8 mg/kg/day (22).

Creatinine clearance (Ccr) in ml/min was calculated from $U_{cr} \times V / S_{cr}$ where U_{cr} = urinary creatinine concentration in mg/ml, S_{cr} = serum creatinine concentration in mg/ml, and V = urinary volume in ml/min (20). The factor 1.73/body surface area in square meter was used to normalize creatinine clearance for average body surface area. The same principle was also applied for the calculation of uric acid clearance.

Table 1. Age, height, initial body weight, and occupation in 8 healthy men on various diets

Subject	Age	Height	Weight	Occupation
	yr	cm	kg	
1	32	163	56.5	Driver
2	34	162	60.1	Lab-personel
3	32	161	70.6	Lab-personel
4	35	154	55.3	Lab-personel
5	30	160	60.2	Lab-personel
6	32	171	71.0	Lab-personel
7	30	168	69.5	Lab-personel
8	32	170	59.3	Lab-personel

Table 2. Menus served during the PF period

Break fast	Lunch and Supper
Mungbean starch tart, jam, and carbonated beverage	Fried mungbean noodle with onion leaf, wax gourd, and carbonated beverage

Table 3. Menus served at various days of VD and OV

Day	Breakfast	Lunch	Supper
1	Boiled rice; fermented soybean curd cake; fried swamp cabbage with soybean paste	Fried noodle with soybean curd cake and collard ^a	Rice; soybean with mixed vegetable soup; fried soybean with chilli paste
2	Rice with fried soybean curd cake and young corn	Rice with fried soybean curd cake, onion and chilli	Rice; soybean curd cake and bamboo shoot curry; fried cabbage with soybean curd cake ^a
3	Boiled rice; fried soybean curd cake and pickled Chinese mustard soup; salted Chinese radish	Fried noodle with soybean curd cake and chilli paste	Rice; mixed vegetable soup with soybean curd cake; fried sugar pea with soybean curd cake ^a
4	Fried Rice ^a	Rice with soybean curry	Rice; fried mungbean noodle with soybean curd cake and vegetable; soybean paste in coconut milk
5	Boiled rice; fried soybean curd cake with Chinese celery; fried pickled Chinese mustard	Fried noodle with texturized protein ^a	Fried collard with soybean curd cake; mushroom and soybean soup
6	Fried rice with fermented soybean curd cake ^a	Fried Chinese radish cake with soybean	Rice; fried sweet and sour soybean curd cake; mungbean noodle and curd sheet soup
7	Boiled rice; fried soybean curd cake; fried soybean sprout with soybean curd cake	Fried wheat noodle with soybean curd cake ^a	Rice; soybean soup; fried cauliflower with soybean curd cake
8	Fried rice with sweet basil and soybean curd cake	Fried noodle with collard and soybean curd cake ^a	Rice; soybean curd cake curry; fried young corn with soybean curd cake
9	Boiled rice; tofu with soybean paste; fried ^a salted Chinese radish	Rice mixing with soybean paste	Rice; mixed vegetable and soybean curry; soybean curd cake with chilli paste
10	Boiled rice; fried soybean; salted cabbage	Fried noodle with soybean curd cake ^a	Rice; soybean curry with vegetable; fried soybean curd cake with young onion

^a

Egg was added during OV period.

Table 4. Menus served at various days of the RD period

Day	Breakfast	Lunch	Supper
1	Boiled rice with chicken	Fried rice noodle with kale and chicken	Vegetable soup Thai style and fried chicken
2	Chicken sandwich	Rice, Fried chicken with hot chili and sweet basil	Rice, fried chicken with ginger, and chinese cabbage soup
3	Rice noodle with chicken soup	Fried rice noodle with Chinese mustard green, soy sauce and chicken	Rice, sweet and sour cucumber, and grilled chicken

CHAPTER III

INFLUENCE OF SOYBEAN COMPARED WITH SOYBEAN-EGG DIETS
ON PROTEIN-CALORIE STATUS

Results

Energy intake

Table 5 shows mean protein, fat, and carbohydrate intake in individual subject during 14 days of the BL period obtained by 24-hr dietary record. Their energy intake and distribution derived from protein, fat, and carbohydrate were then calculated. Their daily energy intake ranged from 1530 to 2115 kcal whereas their energy distribution was 13-18% protein-calories, 31-43% fat-calories, and 40-56% carbohydrate-calories.

Tables 6 and 7 show energy intake for each subject during the PF and VD or OV periods which was held constant according to his mean energy intake plus 1 SD during the BL period. Their protein intake during the PF period contributed only 0.2% of the total energy intake whereas their fat and carbohydrate intake were 30 and 69.7% respectively. During the VD and OV periods, the energy distribution was assigned to be 15% protein-calories, 30% fat-calories, and 55% carbohydrate-calories (Table 7). It should be noted that dietary intake in each subject during the VD and OV periods was isocaloric and isonitrogenous. The difference between these two diets was protein

sources. Soybean and rice were the protein sources of VD at the ratio of 9:1 whereas soybean, egg, and rice were those of OV at the ratio of 7.2:1.8:1.0.

Table 8 shows mean protein, fat, and carbohydrate intake in individual subject during 3 days of the RD period. Their daily energy intake ranged from 1433-1711 kcal whereas their energy distribution was 25-28% protein-calories, 14-17% fat-calories, and 55-61% carbohydrate-calories.

Table 9 shows the mean daily actual energy intake in 7 healthy subjects during the study periods compared with their EER. Their actual energy intake during the BL period was 71% of EER whereas that during PF/VD/OV and RD were 84 and 65%, respectively. Their mean daily actual energy intake and EER expressed by weight basis are shown in Table 10

Protein intake

Table 11 shows individual and mean($\pm$ SEM) daily protein intake derived from soybean and rice in 7 healthy men compared with their safe level of protein intake derived from soybean and rice. The digestibility of total protein in the VD diet was 83% derived from $(0.90 \times 82) + (0.10 \times 93)$ where 0.90 and 0.10 were the fractions of soybean protein and rice protein and 82 and 93 were the percentages of digestibility relative to milk protein (23). Thus the safe level of protein intake in VD was 0.90 g/kg derived

from $0.75 \times 100/83$ where 0.75 g/kg was the safe level of reference protein (23). Their actual protein intake during the VD period ranged from 0.96 to 1.33 g/kg/day which were 107-148% of the safe level of protein intake in VD diet.

Safe level of protein intake derived from soybean, egg and rice during the OV period is shown in Table 12. The digestibility of total protein in the OV diet was 86% where 0.72, 0.18, and 0.10 were the fractions of soybean protein, egg protein, and rice protein, respectively, and 82, 100, and 93 were the percentages of digestibility relative to milk protein (23). Thus the safe level of protein intake in OV was 0.87 g/kg (23). Their actual protein intake during the OV period ranged from 0.96 to 1.33 g/kg/day which were 110-153% of the safe level of protein intake in OV diet.

Table 13 shows that individual and mean daily indispensable amino acid (IDAA) intake in 7 healthy men on VD which were above the safe levels of each IDAA (24). Their IDAA intake constituted 38.6% of total amino acid intake. Their individual and mean daily IDAA intake during the OV period were also higher than the safe levels (Table 14). Their IDAA intake constituted 39.8% of total amino acid intake. Tables 15 and 16 show individual and mean ($\pm$ SEM) daily dispensable amino acid intake in these subjects on VD and OV diet, respectively.

Anthropometric measurement

All of the subjects exhibited significant decrease in body weight after 25 days of metabolic study. The net decrease from the BL period ranged from -0.9 to -4.2 kg with a mean of -2.4 kg being equivalent to -4.3% of the BL period (Table 17). Similar finding was observed for BMI (Table 18). Their body fat also decreased significantly (Tables 19 and 20). Their fat-free mass at the end of the study was lower than that at the BL period but the difference did not reach statistical significance (Table 21). Only subjects 5 and 7 had lost their body muscle mass (Table 22). Their mean upper arm circumferences at the OV and RD periods were significantly lower than that at the BL period (Table 23) whereas there was no significant difference in upper arm muscle circumference (Table 24) and bone-free arm muscle area (Table 25). Their mean waist and hip circumferences decreased during the study and these two parameters during the RD and OV periods were significantly different from the BL period (Tables 26 and 27). There was no striking change in mean waist/hip ratio during the study (Table 28)

Plasma glucose

Table 29 shows that mean plasma glucose level at the RD period was significantly lower than that at the BL period. Plasma glucose levels in each subject were within

normal limit, i.e., less than 140 mg/dL (25), throughout the study. Subjects 4,5, and 7 whose plasma glucose levels were higher than 110 mg/dL at the BL period, showed the decline in plasma glucose levels at the VD, RD, and OV periods.

Serum urea nitrogen

Table 29 shows that mean serum urea nitrogen levels at the VD and RD periods were significantly higher than that at the BL period.

Serum transport proteins

There were no significantly changes in mean serum total protein, albumin, and transferrin levels during the study whereas serum RBP levels at the VD, RD, and OV periods were significantly lower than that at the BL period (Tables 30 and 31)

Urinary protein excretion

Tables 32 shows mean and range of urinary protein excretion in mg/kg/day as well as its coefficient variation (CV) and range expressed as the percentage of mean in 7 healthy individuals during the last 3 days of the BL period. These values during the VD, RD, and OV periods are shown in Tables 33-35. Tables 36 shows urinary protein excretion in these subjects at days 1 and 15 (PF period).

Table 37 shows mean($\pm$ SEM) urinary protein excretions in 7 healthy men on various diets. The mean values of each dietary period were derived from summation of daily urinary protein excretion during each study period of 7 subjects/total urinary samples obtained from these subjects. Thus total urine samples determined were 21 for BL, 14 for PF, 70 for VD and OV, and 21 for RD. Their urinary protein excretions expressed as mg/kg/day or mg/day during the VD, RD, and OV periods were significantly higher than those during the PF period.

Urinary nitrogen excretion

Table 38 shows mean and range of urinary nitrogen excretion in mg/kg/day as well as its CV and range expressed as the percentage of mean in 7 healthy individuals during the last 3 days of the BL period. These values during the VD, RD, and OV periods are shown in (Tables 39-41). Tables 36 shows urinary total nitrogen excretion in these subjects at days 1 and 15 (PF period).

Mean($\pm$ SEM) urinary total nitrogen excretions in 7 healthy men on various diets were also computed using the same principle as described in mean urinary protein excretions (Table 37). Their urinary total nitrogen excretions expressed as mg/kg/day or mg/day during the VD, RD, and OV periods were significantly higher than those during the BL and PF periods. The highest urinary total nitrogen excretion was observed during the RD period which was

significantly higher than during VD and OV periods.

Nitrogen balance

During receiving VD or OV, all of the subjects were in positive nitrogen balance and their nitrogen balances during these two dietary periods were not significantly different (Tables 42 and 43)

Hematological status

Tables 44 - 47 show individual and mean($\pm$ SEM) hemoglobin, hematocrit, RBC count, MCV, MCH, and MCHC in these subjects during the study. Only their hemoglobin and MCHC levels at the VD period were significantly higher than those at the OV and RD periods ($P < 0.025$), respectively. Tables 48-51 show individual and mean($\pm$ SEM) WBC, total lymphocyte, and platelet counts in 7 subjects during the various dietary periods. There were no striking changes in these parameters during the study.

Discussion

In the body, protein is used for the structural formation of cells and tissues, the production of various essential compounds such as enzymes, antibodies, hormones, and protein mediators, for regulating fluid and electrolyte balances as well as blood neutrality. It can also be utilized as an energy source: 1 g of protein provides 4 kcal.

Table 5. Individual and mean($\pm$ SEM) daily energy intake, macronutrient intake, and energy distribution in 7 healthy men during 14 days of BL period

Subject	Energy kcal/day	Protein <----->	Fat g/day	Carbohydrate ----->	Energy distribution		
					Protein ----->	Fat ----->	Carbohydrate ----->
1	1530	54	60	192	14	36	50
2	1680	56	59	231	13	31	56
3	1554	65	59	191	17	34	49
4	1546	71	56	190	18	33	49
5	1681	72	70	191	17	37	46
6	2115	91	102	208	17	43	40
7	1739	62	73	208	14	38	48
Mean $\pm$ SEM	1692 $\pm$ 77	67 $\pm$ 0.4	68 $\pm$ 6.1	202 $\pm$ 5.8	16 $\pm$ 0.7	36 $\pm$ 1.5	48 $\pm$ 1.8

Table 6. Individual and mean($\pm$ SEM) daily energy intake, macronutrient intake, and energy distribution in 7 healthy men on protein-free diet

Subject	Energy kcal/day	Protein <----->	Fat g/day	Carbohydrate ----->	Energy distribution		
					Protein	Fat	Carbohydrate
1	1846	1.24	61.6	321.7	0.2	30.0	69.8
2	1944	1.25	64.8	338.9	0.2	30.0	69.8
3	1814	1.24	60.7	315.9	0.3	30.1	69.6
4	1960	1.25	65.4	341.6	0.2	30.0	69.8
5	1913	1.24	63.9	332.6	0.2	30.1	69.7
6	2430	1.76	81.0	424.0	0.3	30.0	69.7
7	2057	1.25	68.6	358.8	0.2	30.0	69.8
Mean $\pm$ SEM	1995 $\pm$ 78	1.32 $\pm$ 0.07	66.4 $\pm$ 2.6	347.6 $\pm$ 13.8	0.2 $\pm$ 0.02	30.0 $\pm$ 0.02	69.8 $\pm$ 0.03

Table 7. Individual and mean($\pm$ SEM) daily energy intake, macronutrient intake, and energy distribution in 7 healthy men on vegetarian and ovovegetarian diet

Subject	Energy kcal/day	Protein <----- g/day ----->	Fat	Carbohydrate	Energy distribution		
					Protein	Fat	Carbohydrate
					<----- % ----->		
1	1846	69.2	61.5	253.8	15	30	55
2	1944	72.9	64.8	267.2	15	30	55
3	1815	68.0	60.5	249.5	15	30	55
4	1960	73.5	65.3	269.5	15	30	55
5	1913	71.7	63.8	260.1	15	30	55
6	2430	91.1	81.0	334.2	15	30	55
7	2057	77.2	68.6	282.9	15	30	55
Mean±SEM	1995±78	74.7±2.9	66.5±2.6	273.9±10.9	15	30	55

Table 8. Individual and mean($\pm$ SEM) daily energy intake, macronutrient intake, and energy distribution in 7 healthy men on regular diet

Subject	Energy kcal/day	Protein <----->	Fat g/day	Carbohydrate ----->	Energy distribution		
					Protein	Fat	Carbohydrate
1	1433	99	27	198	28	17	55
2	1433	99	27	198	28	17	55
3	1433	99	27	198	28	17	55
4	1433	99	27	198	28	17	55
5	1711	107	28	259	25	15	60
6	1688	107	28	256	25	14	61
7	1711	107	28	259	25	14	61
Mean $\pm$ SEM	1549 $\pm$ 55	102 $\pm$ 1.6	27 $\pm$ 0.2	224 $\pm$ 12.1	27 $\pm$ 0.6	16 $\pm$ 0.6	57 $\pm$ 1.2

Table 9. Individual and mean($\pm$ SEM) daily basal energy expenditure and estimated energy requirement in 7 healthy men and their actual energy intake during consuming various diets

Subject	BEE	EER	Actual energy intake					
			BL		PF/VD/OV		RD	
	<-- kcal/day -->		kcal/day	%EER	kcal/day	%EER	kcal/day	%EER
1	1437	2228	1530	67	1846	83	1433	64
2	1468	2276	1680	74	1944	85	1433	63
3	1621	2512	1554	62	1815	72	1433	57
4	1356	2101	1546	74	1960	93	1433	68
5	1487	2304	1681	73	1913	83	1711	74
6	1676	2598	2115	81	2430	94	1688	65
7	1654	2564	1739	68	2057	80	1711	67
Mean $\pm$ SEM	1528 $\pm$ 46	2369 $\pm$ 72	1692 $\pm$ 77	71 $\pm$ 2.3	1995 $\pm$ 78	84 $\pm$ 2.8	1549 $\pm$ 55	65 $\pm$ 2.0

Table 10. Individual and mean($\pm$ SEM) basal energy expenditure and estimated energy requirement in 7 healthy men and their actual energy intake expressed by weight basis during consuming various diets

Subject	BEE	RER	Actual energy intake		
			BL	PF/VD/OV	RD
<----- kcal/kg/day ----->					
1	25.4	39.4	27.1	32.7	25.4
2	24.4	37.8	28.0	32.3	23.8
3	23.0	35.6	22.0	25.7	20.3
4	24.5	38.0	28.0	35.4	25.9
5	24.7	38.3	27.9	31.8	28.4
6	23.6	36.6	29.8	34.2	23.8
7	23.8	36.9	25.0	29.6	24.6
Mean±SEM	24.2±0.3	37.5±0.5	26.8±1.0	31.7±1.2	24.2±1.0

Table 11. Individual and mean($\pm$ SEM) daily protein intake derived from soybean and rice in 7 healthy men compared with their safe level of protein intake during the VD period

Subject	Protein intake				
	Safe level	Soybean	Rice	Total	
	g/kg/day	g/day	g/day	g/kg/day	% of safe level
1	0.90	62.1	7.1	69.2	1.22
2	0.90	65.5	7.4	72.9	1.21
3	0.90	61.0	7.0	68.0	0.96
4	0.90	66.1	7.4	73.5	1.33
5	0.90	64.5	7.2	71.7	1.19
6	0.90	81.7	9.4	91.1	1.28
7	0.90	69.1	8.1	77.2	1.11
Mean $\pm$ SEM	0.90	67.1 $\pm$ 2.6	7.6 $\pm$ 0.3	74.8 $\pm$ 2.9	1.18 $\pm$ 0.04
					132 $\pm$ 5.1

Table 12. Individual and mean($\pm$ SEM) daily protein intake derived from soybean, egg, and rice in 7 healthy men compared with their safe level of protein intake during the OV period

Subjects	Protein intake						
	Safe level	Soybean	Egg	Rice		Total	% of safe level
	g/kg/day	g/day	g/day	g/day	g/day	g/kg/day	
1	0.87	49.9	12.4	6.9	69.2	1.22	140
2	0.87	52.4	13.1	7.3	72.9	1.21	139
3	0.87	49.0	12.2	6.8	68.0	0.96	110
4	0.87	52.9	13.2	7.3	73.5	1.33	153
5	0.87	51.5	13.0	7.2	71.7	1.19	137
6	0.87	65.3	16.4	9.4	91.1	1.28	147
7	0.87	55.2	14.0	8.0	77.2	1.11	128
Mean $\pm$ SEM	0.87	53.7 $\pm$ 2.1	13.5 $\pm$ 0.5	7.6 $\pm$ 0.3	74.8 $\pm$ 2.9	1.18 $\pm$ 0.04	136 $\pm$ 5.3

Table 13. Individual and mean($\pm$ SEM) daily indispensable amino acid intake in 7 healthy men on vegetarian diet compared with the safe levels

subject	His	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Try	Val	mg/kg/day		IDAA
										<----->	<----->	%total /
1	33.9	58.8	101.1	77.1	34.4	103.3	50.1	15.4	60.6	484.6	38.6	38.6
2	33.4	58.0	100.0	76.0	20.3	101.5	49.2	15.2	59.5	478.0	38.6	38.6
3	25.8	44.8	77.0	58.6	26.3	78.5	38.2	11.6	46.1	369.4	38.6	38.6
4	36.4	62.9	108.4	82.4	36.9	110.4	53.7	16.4	64.7	519.2	38.6	38.6
5	33.4	57.8	99.5	75.7	33.9	101.4	49.2	13.8	59.6	476.8	38.6	38.6
6	35.3	61.5	105.9	80.5	36.2	107.8	52.4	16.1	63.2	507.2	38.6	38.6
7	31.1	54.0	92.8	70.6	31.8	94.6	44.8	14.2	55.5	444.8	38.6	38.6
mean $\pm$ SEM	32.8 $\pm$ 1.3	56.8 $\pm$ 2.3	97.8 $\pm$ 4.0	63.8 $\pm$ 9.6	31.4 $\pm$ 2.3	99.6 $\pm$ 4.0	48.2 $\pm$ 2.0	14.7 $\pm$ 0.6	58.4 $\pm$ 2.3	468.6 $\pm$ 18.8	38.6	38.6
safe level mg/kg/day	-	23	39	30	15	39	15	6	20	187		
of safe level -	247	251	213	209	255	321	245	292	233			

Table 14. Individual and mean($\pm$ SEM) daily indispensable amino acid intake in 7 healthy men on ovo-vegetarian diet compared with the safe levels

Subject	His	Ile	Leu	Lys	Met+Cys	Phe+Tyr	Thr	Try	Val	mg/kg/day	
										<-----	>-----
1	33.7	63.1	104.4	79.1	41.7	108.1	53.0	16.5	66.8	511.1	39.8
2	33.0	61.7	101.9	77.3	40.5	105.7	51.5	16.0	65.3	498.3	39.8
3	25.7	48.1	79.5	60.2	31.6	81.1	40.3	12.5	48.1	388.8	39.8
4	36.4	68.3	112.8	85.5	44.9	117.0	57.2	17.7	72.2	550.9	39.8
5	32.8	61.5	101.6	77.0	40.5	105.3	51.5	16.0	65.2	497.3	39.8
6	35.3	66.0	109.1	82.6	43.5	113.2	55.3	17.2	70.0	533.8	39.8
7	31.1	58.2	96.3	72.9	38.4	99.8	48.8	15.2	61.9	470.1	39.8
mean $\pm$ SEM	32.6 $\pm$ 1.3	61.0 $\pm$ 2.5	100.8 $\pm$ 4.1	76.4 $\pm$ 3.1	40.2 $\pm$ 1.6	104.3 $\pm$ 4.4	51.1 $\pm$ 2.1	13.7 $\pm$ 2.2	64.2 $\pm$ 3.0	492.9 $\pm$ 20.0	39.8
safe level mg/kg/day	-	23	39	30	15	39	15	6	20	187	
of safe level -	265	258	255	268	267	341	228	321	263		

Table 15. Individual and mean($\pm$ SEM) daily dispensable amino acid intake in 7 healthy men on vegetarian diet

Subject	Cys	Arg	Ala	Asp	Glu	Gly	Pro	Ser
<----- mg/kg/day ----->								
1	16.2	88.7	58.7	145.9	242.9	53.6	67.7	63.3
2	16.0	87.2	57.8	143.6	239.0	52.7	66.6	62.3
3	12.3	67.4	44.7	110.9	184.8	40.7	51.5	48.1
4	17.4	94.9	62.8	156.1	259.9	57.3	72.5	67.7
5	16.0	87.1	57.7	143.4	238.7	52.6	66.6	62.2
6	17.0	92.7	61.4	152.5	253.9	56.0	70.8	66.2
7	14.9	81.4	53.9	133.8	222.9	49.1	62.1	58.1
Mean $\pm$ SEM	15.7 $\pm$ 0.6	85.6 $\pm$ 3.4	56.7 $\pm$ 2.3	140.9 $\pm$ 5.7	234.6 $\pm$ 9.4	51.7 $\pm$ 2.1	65.4 $\pm$ 2.6	61.1 $\pm$ 2.5

Table 16. Individual and mean($\pm$ SEM) daily dispensable amino acid intake in 7 healthy men on ovovegetarian diet

Subject	Cys	Arg	Ala	Asp	Glu	Gly	Pro	Ser
mg/kg/day								
1	18.8	88.7	62.4	143.8	232.0	52.4	65.5	70.0
2	18.4	86.6	61.0	140.4	226.4	51.1	64.0	68.3
3	14.3	67.5	47.6	109.5	176.6	39.9	49.9	53.3
4	20.4	95.9	67.5	155.4	250.6	56.6	70.8	75.6
5	18.4	86.4	60.8	140.0	225.8	51.0	63.8	68.2
6	19.7	92.8	65.4	150.2	242.4	54.8	68.5	73.2
7	17.4	81.9	57.7	132.5	213.8	48.3	60.4	64.6
Mean $\pm$ SEM	18.2 $\pm$ 0.7	85.7 $\pm$ 3.5	60.3 $\pm$ 2.4	138.8 $\pm$ 5.6	223.9 $\pm$ 9.1	50.6 $\pm$ 2.0	63.3 $\pm$ 2.6	67.6 $\pm$ 2.7

Table 17. Individual and mean($\pm$ SEM) body weight in 7 healthy men on various diets

Subjects	Body weight				Net change	
	BL	VD	RD	OV	OV-BL	
	<----- kg ----->				kg	% BL
1	56.5	55.1	54.9	54.0	-2.5	-4.4
2	60.1	59.0	59.8	58.2	-1.9	-3.2
3	70.6	71.2	70.7	69.7	-0.9	-1.3
4	55.3	54.7	54.0	53.0	-2.3	-4.2
5	60.2	58.1	57.4	56.1	-4.1	-6.8
6	71.0	69.4	69.1	68.0	-3.0	-4.2
7	69.5	67.0	66.3	65.3	-4.2	-6.0
Mean $\pm$ SEM	63.3 $\pm$ 2.6	62.1 $\pm$ 2.6	61.7 $\pm$ 2.6	60.6 $\pm$ 2.6	-2.4 $\pm$ 0.6	-4.3 $\pm$ 0.7
VD, RD, OV < BL:	a ¹	a ²	a ²	a ^{1, b, c}		
	P<0.001,	P<0.025,	OV < VD: P<0.001,	OV < RD: P<0.001		

Table 18. Individual and mean($\pm$ SEM) body mass index in 7 healthy men on various diets

Subjects	Body mass index				Net change	
	BL	VD	RD	OV	OV-BL	
	$\langle \text{---} \text{kg/m}^2 \text{---} \rangle$				kg/m^2	%BL
1	21.1	20.6	20.5	20.2	-0.9	-4.3
2	22.9	22.5	22.8	22.2	-0.7	-3.0
3	27.4	26.5	26.3	25.9	-1.5	-5.5
4	23.3	23.1	22.8	22.3	-1.0	-4.3
5	23.5	22.7	22.4	21.9	-1.6	-6.8
6	24.3	23.7	23.6	23.3	-1.0	-4.1
7	24.6	23.7	23.5	23.1	-1.5	-6.1
Mean $\pm$ SEM	23.9 ± 0.7	23.2 ± 0.7	23.1 ± 0.6	22.7 ± 0.6	-1.2 ± 0.1	-4.9 ± 0.5
RD, VD, OV:	$P < 0.001$, a_1	$P < 0.025$, a_2	$P < 0.001$, a_2	$P < 0.001$, a_1, b, c	$P < 0.001$, b	$P < 0.001$, c

Table 19. Mean($\pm$ SEM) 4 skinfold thicknesses and percentage of body fat in 7 healthy men on various diets

Diets	Skinfold				Body fat
	Triceps	Biceps	Subscapular	Suprailiac	Sum
	mm				%
BL	13.9 $\pm$ 0.9	5.4 $\pm$ 0.3	26.3 $\pm$ 2.6	39.0 $\pm$ 4.2	85.7 $\pm$ 5.8
VD	12.6 $\pm$ 0.8	5.4 $\pm$ 0.5	24.8 $\pm$ 2.5	37.1 $\pm$ 3.7	81.3 $\pm$ 6.1
RD	12.1 $\pm$ 0.9	5.4 $\pm$ 0.5	24.2 $\pm$ 2.3	36.4 $\pm$ 3.2	78.2 $\pm$ 5.2
OV	11.6 $\pm$ 0.8	5.2 $\pm$ 0.4	22.8 $\pm$ 2.4	35.2 $\pm$ 3.0	74.8 $\pm$ 4.8

VD, RD, OV < BL: $P < 0.005$, $P < 0.01$, $P < 0.025$, $P < 0.05$
 OV < VD : $P < 0.01$, $P < 0.025$, $P < 0.05$
 OV < RD : $P < 0.01$, $P < 0.025$, $P < 0.05$

Table 20. Individual and mean($\pm$ SEM) body fat in 7 healthy men on various diets

Subjects	Body fat				Net change	
	BL	VD	RD	OV	OV-BL	
	<----- kg ----->				kg	% BL
1	15.8	14.9	14.2	13.9	-1.9	-12.0
2	16.5	16.1	15.9	15.2	-1.3	-7.9
3	19.4	19.7	19.2	18.8	-0.6	-3.1
4	15.4	14.4	13.9	13.4	-2.0	-13.0
5	16.4	15.6	15.4	15.0	-1.4	-8.5
6	21.4	20.2	19.8	18.8	-2.6	-12.1
7	15.6	14.2	14.6	14.0	-1.6	-10.2
Mean $\pm$ SEM	17.2 $\pm$ 0.9	16.5 $\pm$ 0.9	16.2 $\pm$ 0.9	15.6 $\pm$ 0.9	-1.6 $\pm$ 0.2	-9.5 $\pm$ 1.3
VD, RD, OV < BL:	a1	a2	a3	a1, b, c		
	P<0.001,	P<0.005,	P<0.025			
OV < VD	b	c				
	: P<0.005,	OV < RD:	P<0.001			

Table 21. Individual and mean($\pm$ SEM) fat-free mass in 7 healthy men on various diets

Subjects	Fat-free mass				Net change	
	BL	VD	RD	OV	OV-BL	
	<----- kg ----->				kg	% BL
1	40.7	40.2	40.7	40.1	-0.6	1.5
2	43.6	42.9	43.9	43.0	-0.6	1.4
3	51.2	51.5	51.5	51.0	-0.2	0.4
4	39.9	40.3	40.1	39.6	-0.3	0.8
5	43.8	42.5	42.0	41.1	-2.7	6.0
6	49.6	49.2	49.3	49.2	-0.4	0.8
7	53.9	52.8	51.7	51.3	-2.6	4.8
Mean $\pm$ SEM	46.1 $\pm$ 2.1	45.6 $\pm$ 2.0	47.3 $\pm$ 2.1	46.7 $\pm$ 2.2	-1.0 $\pm$ 0.4	-2.2 $\pm$ 0.8

Table 22. Individual and mean($\pm$ SEM) body muscle mass in 7 healthy men on various diets

Subjects	Body muscle mass				Net change	
	BL	VD	RD	OV	OV-BL	% BL
	<----- kg ----->				kg	
1	19.2	19.9	20.2	20.1	0.9	4.6
2	22.8	24.1	24.3	24.6	1.8	7.9
3	23.4	22.8	23.4	23.7	0.3	1.3
4	22.2	22.4	21.9	22.3	0.1	0.4
5	22.0	22.3	22.0	21.7	-0.3	-1.4
6	25.0	24.9	24.5	25.5	0.5	2.0
7	24.5	24.4	23.4	23.3	-1.2	-4.9
Mean $\pm$ SEM	22.7 $\pm$ 0.7	23.0 $\pm$ 0.6	22.8 $\pm$ 0.6	23.6 $\pm$ 0.7	0.3 $\pm$ 0.4	1.4 $\pm$ 1.6

Table 23. Individual and mean($\pm$ SEM) upper arm circumference in 7 healthy men on various diets

Subjects	Upper arm circumference			
	BL	VD	RD	OV
	<----- cm ----->			
1	26.4	26.4	26.4	26.5
2	30.0	29.5	29.7	29.8
3	29.3	29.2	28.9	28.9
4	28.4	28.3	27.8	27.9
5	29.5	29.5	29.5	28.9
6	30.2	29.8	29.5	29.4
7	30.4	29.6	29.0	29.0
Mean $\pm$ SEM	29.2 $\pm$ 0.5	28.9 $\pm$ 0.5	28.7 $\pm$ 0.4 ^{a2}	28.6 $\pm$ 0.4 ^{a1}
RD,OV < BL: ^{a1} P<0.025, ^{a2} P<0.05				

Table 24. Individual and mean($\pm$ SEM) upper arm muscle circumference in 7 healthy men on various diets

Subjects	Upper arm muscle circumference			
	BL	VD	RD	OV
	<----- cm ----->			
1	22.8	23.3	23.4	23.4
2	24.9	25.6	25.8	25.9
3	25.3	24.9	25.3	25.5
4	25.2	25.3	25.0	25.3
5	24.7	24.8	24.7	24.5
6	25.4	25.3	25.2	25.6
7	25.4	25.3	24.8	24.7
Mean $\pm$ SEM	24.8 $\pm$ 0.3	24.9 $\pm$ 0.3	24.9 $\pm$ 0.3	25.0 $\pm$ 0.3

Table 26. Individual and mean($\pm$ SEM) waist circumference in 7 healthy men on various diets

Subjects	Waist circumference			
	BL	VD	RD	OV
	<----- cm ----->			
1	78.5	77.5	76.4	76.2
2	76.0	76.5	75.0	74.5
3	85.0	81.5	81.5	78.6
4	77.5	76.8	75.7	74.6
5	74.5	73.5	72.5	71.5
6	82.5	81.4	80.2	79.5
7	76.5	75.8	75.9	75.4
Mean $\pm$ SEM	78.6 $\pm$ 1.4	77.6 $\pm$ 1.1	76.7 $\pm$ 1.2 ^{a,b}	75.8 $\pm$ 1.0 ^{a,b,c}

^a RD,OV < BL: P<0.05, ^b RD,OV < VD: P<0.025, ^c OV < RD: P<0.05

Table 27. Individual and mean($\pm$ SEM) hip circumference in 7 healthy men on various diets

Subjects	Hip circumference			
	BL	VD	RD	OV
	cm			
1	80.0	79.8	79.2	78.5
2	74.5	75.2	74.2	75.2
3	86.5	80.6	81.5	80.5
4	78.0	77.5	77.0	76.5
5	78.4	76.7	77.0	76.0
6	85.5	84.2	83.5	82.7
7	78.5	77.8	77.6	76.9
Mean $\pm$ SEM	80.2 $\pm$ 1.6	78.8 $\pm$ 1.1	78.6 $\pm$ 1.2 ^a	78.0 $\pm$ 1.0 ^{a,b}
RD,OV < BL: P<0.05, ^a OV < VD: P<0.025 ^b				

Table 28. Individual and mean($\pm$ SEM) waist/hip ratio
in 7 healthy men on various diets

Subjects	Waist/hip ratio			
	BL	VD	RD	OV
1	0.98	0.97	0.96	0.97
2	1.02	1.02	1.01	0.99
3	0.98	1.01	1.00	0.98
4	0.99	0.99	0.98	0.98
5	0.95	0.96	0.94	0.94
6	0.96	0.97	0.96	0.96
7	0.97	0.97	0.98	0.98
Mean $\pm$ SEM	0.98 $\pm$ 0.008	0.98 $\pm$ 0.009	0.98 $\pm$ 0.009	0.97 $\pm$ 0.006

Table 29. Individual and mean($\pm$ SEM) plasma glucose and serum urea nitrogen in 7 healthy men on various diets

Subject	Plasma glucose			Serum urea nitrogen				
	BL	VD	RD	OV	BL	VD	RD	OV
mg/dL								
1	108	107	107	108	11.5	13.5	15.0	13.8
2	87	98	83	95	14.9	17.7	18.9	15.2
3	104	103	97	108	15.0	18.6	15.5	14.2
4	122	105	116	107	18.2	18.6	20.2	15.5
5	114	111	103	95	13.2	16.9	15.2	16.0
6	110	112	103	108	17.7	18.2	18.6	18.4
7	116	112	105	108	18.9	17.9	19.4	18.2
Mean±SEM	109 ₄	107 ₂	102 ₄	104 ₂	15.6 _{1.0}	17.4 _{0.7}	17.6 _{0.8}	15.9 _{0.7}
RD < BL: P<0.005, VD,RD > BL: P<0.025, P<0.05								

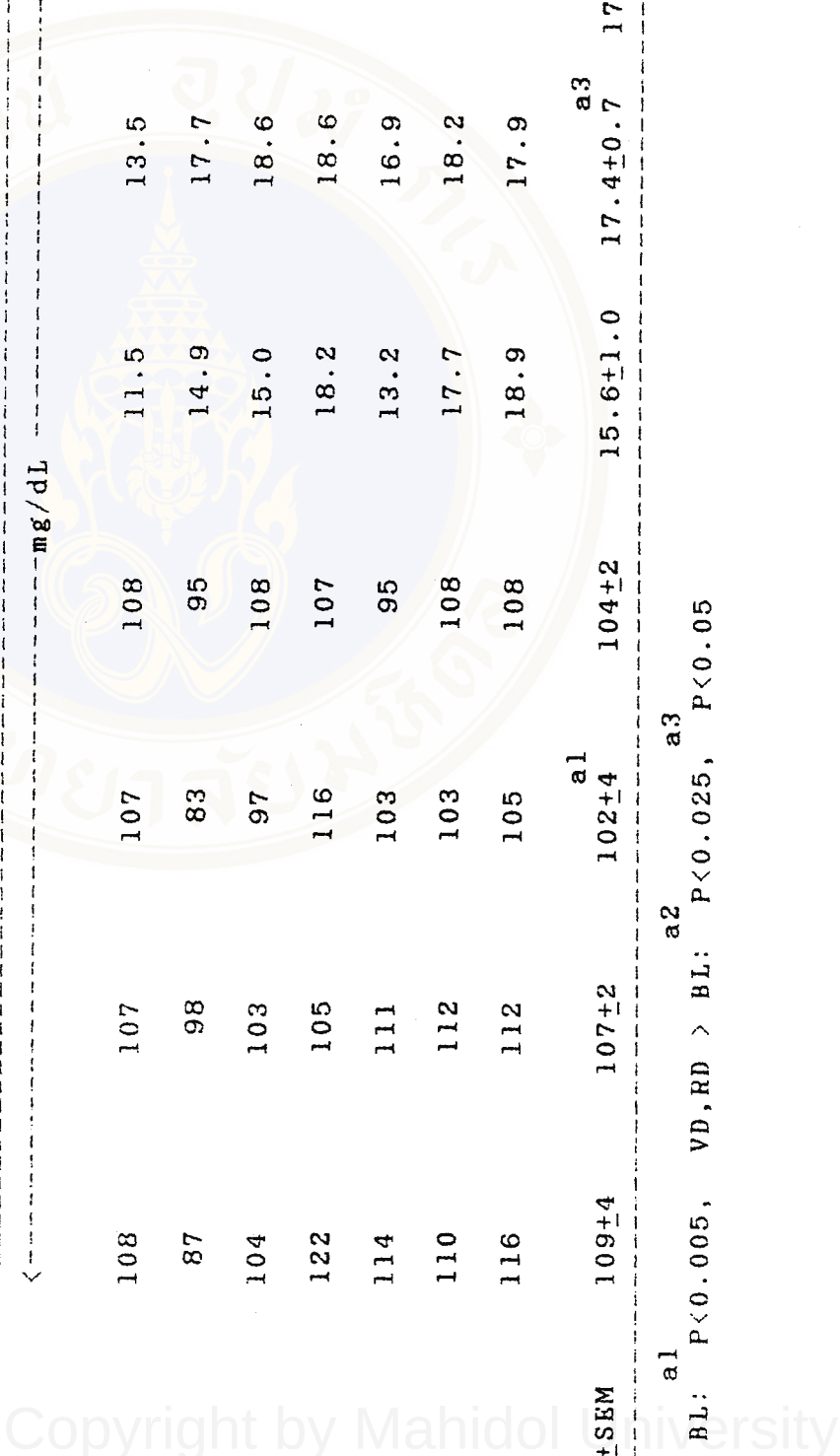


Table 30. Individual and mean($\pm$ SEM) serum total protein and albumin in 7 healthy men on various diets

Subject	Serum total protein				Serum albumin			
	BL	VD	RD	OV	BL	VD	RD	OV
	g/L							
1	70.7	67.9	70.7	73.9	55.5	54.8	57.1	58.1
2	74.3	73.2	73.2	71.4	51.6	49.1	54.1	52.6
3	85.7	68.9	68.9	71.4	55.4	51.4	55.5	56.8
4	78.6	75.0	71.4	73.9	57.6	54.4	55.8	56.9
5	79.6	85.7	77.5	77.5	55.5	57.6	57.2	50.8
6	78.6	74.3	75.4	75.4	55.2	55.4	57.2	56.2
7	75.0	75.0	72.1	67.1	55.5	62.6	56.5	56.9
Mean $\pm$ SEM	77.5 $\pm$ 1.8	74.3 $\pm$ 2.2	72.7 $\pm$ 1.1	72.9 $\pm$ 1.3	55.2 $\pm$ 0.7	55.0 $\pm$ 1.6	56.2 $\pm$ 0.4	55.5 $\pm$ 1.0

Table 31. Individual and mean($\pm$ SEM) serum transferrin and retinol-binding protein 7 healthy men on various diets

Subject	Serum transferrin				Serum retinol-binding protein			
	BL	VD	RD	OV	BL	VD	RD	OV
	mg/dL				mg/L			
1	171	210	210	162	55.0	45.0	43.5	45.0
2	186	162	204	210	41.0	35.5	38.0	38.0
3	282	171	210	240	49.5	36.5	35.5	39.0
4	210	240	282	306	57.0	36.5	35.5	38.0
5	231	171	156	150	40.0	31.5	30.5	28.0
6	231	177	210	240	60.5	44.5	46.0	49.5
7	204	219	177	177	43.0	34.0	31.5	26.3
Mean $\pm$ SEM	216 $\pm$ 14	193 $\pm$ 11	207 $\pm$ 15	212 $\pm$ 21	49.4 $\pm$ 3.1	37.6 $\pm$ 2.0	37.2 $\pm$ 2.2	37.7 $\pm$ 3.2

VD, RD, OV < BL: ^a P<0.001

Table 32. Urinary protein excretion in 7 healthy individuals during the last 3 days of the baseline period

Subject	Urinary protein			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	2.37	6	2.20-2.48	93-105
2	6.91	76	1.85-12.30	23-178
3	0.86	60	0.31-1.32	36-153
4	2.77	48	1.68-4.24	61-153
5	1.20	34	0.74-1.46	62-122
6	1.25	68	0.29-1.89	23-151
7	0.85	61	0.27-1.27	32-149

Table 33. Urinary protein excretion in 7 healthy individuals during the vegetarian period (10 days)

Subject	Urinary protein			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	3.38	41	1.72-6.43	51-190
2	1.79	33	0.92-2.95	51-164
3	0.93	40	0.34-1.36	37-146
4	2.51	14	2.03-3.33	81-132
5	1.31	21	0.91-1.65	69-126
6	1.50	31	0.95-2.54	63-169
7	1.86	24	1.09-2.58	59-139

Table 34. Urinary protein excretion in 7 healthy individuals during the regular period (3 days)

Subject	Urinary protein			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	3.85	7	3.53-4.09	92-106
2	1.51	9	1.40-1.67	93-111
3	1.55	50	0.85-2.38	55-153
4	2.16	38	1.43-3.06	66-141
5	1.22	14	1.02-1.34	84-110
6	1.55	19	1.33-1.88	86-121
7	1.48	20	1.15-1.73	77-116

Table 35. Urinary protein excretion in 7 healthy individuals during the ovovegetarian period (10 days)

Subject	Urinary protein			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	4.58	28	2.96-6.81	64-149
2	1.71	23	1.09-2.29	64-134
3	1.23	33	0.56-1.72	45-140
4	1.90	24	1.29-2.62	68-138
5	1.96	60	0.65-4.08	33-208
6	2.04	56	0.76-3.95	37-194
7	1.69	59	0.94-4.40	55-260

Table 36. Individual and mean($\pm$ SEM) urinary protein and total nitrogen excretions in 7 healthy men during the protein-free period (days 1 and 15)

Subject	Protein		Total nitrogen	
	Day 1	Day 15	Day 1	Day 15
<----- mg/kg/day ----->				
1	2.69	3.11	40.2	52.5
2	0.94	1.60	89.8	72.6
3	0.50	1.04	136.3	108.1
4	1.35	0.81	88.8	130.0
5	0.84	0.67	41.5	69.9
6	0.99	0.84	130.0	116.8
7	1.07	1.04	84.9	91.9
Mean $\pm$ SEM	1.20 $\pm$ 0.27	1.30 $\pm$ 0.32	87.4 $\pm$ 14.3	91.7 $\pm$ 10.6

Table 37. Mean($\pm$ SEM) urinary protein and total nitrogen excretions in 7 healthy men on various diets

Diet Samples	Protein		Total nitrogen	
	mg/kg/day	mg/day	mg/kg/day	mg/day
BL 21	2.32 $\pm$ 0.59 ^{b1}	141 $\pm$ 35 ^{b1}	94.2 $\pm$ 7.0	594 $\pm$ 46
PF 14	1.25 $\pm$ 0.20	76 $\pm$ 11	90.4 $\pm$ 8.5	571 $\pm$ 64
VD 70	1.90 $\pm$ 0.12 ^{b5}	116 $\pm$ 6 ^{b4}	119.2 $\pm$ 4.3 ^{a2,b4}	732 $\pm$ 25 ^{a3,b5}
RD 21	1.90 $\pm$ 0.21 ^{b6}	115 $\pm$ 11 ^{b5}	151.1 $\pm$ 9.6 ^{a1,b1,c2}	928 $\pm$ 56 ^{a3,b1,c1}
OV 70	2.16 $\pm$ 0.16 ^{b3}	128 $\pm$ 9 ^{b2}	118.8 $\pm$ 4.5 ^{a3,b4,d2}	722 $\pm$ 26 ^{a4,b5,d1}
VD, RD, OV > BL: ^{a1} P<0.0001, ^{a2} P<0.0005, ^{a3} P<0.01, ^{a4} P<0.025 ^{b1} ^{b2} ^{b3} ^{b4} ^{b5}				
BL, VD, RD, OV > PF: ^{b6} P<0.0005, P<0.001, P<0.005, P<0.01, P<0.025, ^{b6} P<0.05				
RD > VD	^{c1} P<0.001,	^{c2} P<0.005		
OV < RD	^{d1} P<0.001,	^{d2} P<0.005		

Table 38. Urinary total nitrogen excretion in 7 healthy individuals during the last 3 days of the baseline period

Subject	Urinary total nitrogen			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	88.5	11	81.1-99.8	92-113
2	79.1	14	67.9-90.8	86-115
3	108.8	35	68.7-143.8	63-132
4	135.3	16	121.3-160.0	90-118
5	75.6	6	70.6-78.6	93-104
6	115.3	28	89.0-151.7	77-132
7	72.7	45	31.4-76.4	55-134

Table 39. Urinary total nitrogen excretion in 7 healthy individuals during the vegetarian period (10 days)

Subjects	Urinary total nitrogen			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	114.8	18	73.9-142.6	64-124
2	120.9	23	80.2-169.0	66-140
3	109.3	19	80.1-146.6	73-134
4	173.0	10	140.8-203.8	81-118
5	102.2	39	66.8-192.6	65-188
6	111.3	32	48.4-158.0	44-142
7	102.6	32	51.8-152.1	50-148

Table 40. Urinary total nitrogen excretion in 7 healthy individuals during the regular period (3 days)

Subject	Urinary total nitrogen			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	161.0	9	151.2-177.6	94-110
2	149.4	26	105.2-180.6	70-121
3	155.3	10	137.1-165.5	88-106
4	212.6	24	164.3-264.6	77-124
5	88.5	42	45.5-113.9	51-129
6	135.6	5	127.3-141.2	94-104
7	155.3	23	129.3-196.2	83-126

Table 41. Urinary total nitrogen excretion in 7 healthy individuals during the ovovegetarian period (10 days)

Subject	Urinary total nitrogen			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	109.8	13	83.2-127.8	76-116
2	113.1	25	41.1-140.2	36-124
3	113.1	22	85.4-166.6	75-147
4	156.4	22	100.6-214.0	64-137
5	104.6	54	73.1-233.7	71-223
6	132.4	22	109.3-208.1	82-157
7	108.6	19	79.8-143.0	73-132

Table 42. Individual and mean($\pm$ SEM) nitrogen balance in 7 healthy men on vegetarian diet

Subject	Nitrogen intake	Nitrogen output			Nitrogen balance		
		Urinary	Fecal	Miscellaneous	g/5 days	g/day	mg/kg/day
<----- g/5 days ----->							
1	55.38	32.02	10.90	2.20	10.26	2.05	37.20
2	58.30	36.35	8.06	2.35	11.54	2.31	39.15
3	54.66	35.86	10.24	2.85	5.71	1.14	16.01
4	58.80	46.36	8.02	2.20	2.22	0.44	8.04
5	57.40	32.62	6.80	2.30	15.68	3.14	54.04
6	72.91	42.07	10.14	2.80	17.90	3.58	51.58
7	67.72	38.60	10.00	2.70	16.42	3.28	48.96
Mean±SEM	60.74±2.60	37.70±1.94	9.16±0.58	2.47±0.10	11.39±2.20	2.28±0.44	36.42±6.77

Table 43. Individual and mean($\pm$ SEM) nitrogen balance in 7 healthy men on ovovegetarian diet

Subject	Nitrogen intake	Nitrogen output			Nitrogen balance		
		Urinary	Miscellaneous		g/day	mg/kg/day	
			Fecal	<----->			
		g/5 days					
1	55.38	29.55	7.61	2.20	16.02	3.20	58.36
2	58.30	30.71	7.56	2.40	17.63	3.53	58.96
3	54.66	42.23	5.12	2.82	4.50	0.90	12.73
4	58.80	43.05	12.08	2.15	1.52	0.30	5.63
5	57.40	37.09	6.12	2.30	11.89	2.38	41.43
6	72.91	49.38	16.54	2.75	4.24	0.85	12.27
7	67.72	38.33	5.19	2.65	21.55	4.31	65.01
Mean $\pm$ SEM	60.74 $\pm$ 2.60	38.62 $\pm$ 2.65	8.60 $\pm$ 1.60	2.47 $\pm$ 0.10	11.05 $\pm$ 2.92	2.21 $\pm$ 0.58	36.34 $\pm$ 9.67

Table 44. Individual and mean($\pm$ SEM) hemoglobin, hematocrit, red blood cell count, MCV, MCH, and MCHC in 7 healthy men during the baseline period

Subject	Hemoglobin g/dL	Hematocrit %	Red blood cell count $\frac{10^{12}}{L}$	MCV fL	MCH pg	MCHC g/L
1	14.5	42.0	8.76	48	16.6	345
2	15.2	44.1	5.01	88	30.3	344
3	16.3	49.4	5.38	92	30.3	330
4	16.5	47.8	5.21	92	31.7	345
5	16.8	49.5	5.16	96	32.5	339
6	16.8	49.8	5.46	91	30.8	338
7	15.9	48.0	5.63	86	28.2	331
Mean $\pm$ SEM	16.0 $\pm$ 0.3	47.2 $\pm$ 1.1	5.80 $\pm$ 0.50	85 $\pm$ 6	28.6 $\pm$ 2.1	339 $\pm$ 2

Table 45. Individual and mean($\pm$ SEM) hemoglobin, hematocrit, red blood cell count, MCV, MCH, and MCHC in 7 healthy men during the vegetarian period

Subject	Hemoglobin g/dL	Hematocrit %	Red blood cell count $\times 10^{12}$ /L	MCV fL	MCH pg	MCHC g/L
1	14.2	43.3	4.91	88	28.9	328
2	15.6	46.3	4.67	99	33.5	338
3	16.4	46.2	4.61	100	35.5	354
4	16.1	45.1	5.19	87	31.0	356
5	15.5	47.7	5.40	88	28.7	325
6	17.2	48.6	5.59	87	30.7	353
7	15.8	45.7	5.24	87	30.1	341
Mean $\pm$ SEM	15.8 $\pm$ 0.3	46.1 $\pm$ 0.6	5.09 $\pm$ 0.14	91 $\pm$ 2	31.2 $\pm$ 0.9	342 $\pm$ 5

Table 46. Individual and mean($\pm$ SEM) hemoglobin, hematocrit, red blood cell count, MCV, MCH, and MCHC in 7 healthy men during the regular period

Subject	Hemoglobin g/dL	Hematocrit %	Red blood cell count $\frac{10^{12}}{L}$	MCV fL	MCH pg	MCHC g/L
1	15.0	47.0	6.32	75	23.8	320
2	14.8	45.6	5.12	89	29.0	338
3	16.2	47.0	5.38	87	30.1	345
4	15.7	44.0	4.92	89	31.8	356
5	15.0	47.9	5.20	92	28.8	313
6	16.5	48.1	4.89	98	33.7	343
7	17.0	49.1	6.14	80	27.7	346
Mean $\pm$ SEM	15.7 $\pm$ 0.3	47.0 $\pm$ 0.6	5.42 $\pm$ 0.22	87 $\pm$ 3	29.3 $\pm$ 1.2	337 $\pm$ 6

Table 47. Individual and mean($\pm$ SEM) hemoglobin, hematocrit, red blood cell count, MCV, MCH, and MCHC in 7 healthy men during the ovo-vegetarian period

Subject	Hemoglobin g/dL	Hematocrit %	Red blood cell count 10^{12} /L	MCV fL	MCH pg	MCHC g/L
1	14.1	43.8	6.24	70	22.6	321
2	15.0	46.1	5.29	87	28.4	326
3	15.5	46.9	5.50	86	28.2	330
4	15.0	44.4	5.25	85	28.5	337
5	14.4	47.7	4.91	97	29.4	303
6	17.2	49.1	5.29	93	32.6	351
7	15.6	43.6	4.74	92	32.8	357
Mean $\pm$ SEM	15.2 $\pm$ 0.4	45.9 $\pm$ 0.8	5.3 $\pm$ 0.2	87 $\pm$ 3	28.9 $\pm$ 1.3	332 $\pm$ 7

Table 48. Individual and mean($\pm$ SEM) white blood cell, total lymphocyte, and platelet counts in 7 healthy men during the baseline period

Subject	White blood cell	Total lymphocyte	Platelet
		$\frac{9}{10} / L$	
1	11.6	4.29	235
2	7.3	3.28	154
3	7.3	2.54	204
4	6.3	2.84	164
5	7.3	3.50	250
6	6.1	2.20	176
7	9.1	2.55	230
Mean $\pm$ SEM	7.8 $\pm$ 0.7	3.00 $\pm$ 0.3	202 $\pm$ 14

Table 49. Individual and mean($\pm$ SEM) white blood cell, total lymphocyte, and platelet counts in 7 healthy men during the vegetarian period

Subject	White blood cell	Total lymphocyte	Platelet
$\xrightarrow{\quad\quad\quad 9 \quad\quad\quad} 10^9 /L \xrightarrow{\quad\quad\quad}$			
1	10.4	3.33	224
2	7.6	2.70	148
3	6.8	2.72	198
4	6.3	3.06	225
5	6.8	2.38	235
6	5.1	1.94	198
7	6.2	2.36	262
Mean $\pm$ SEM	7.0 $\pm$ 0.6	2.64 $\pm$ 0.18	213 $\pm$ 14

Table 50. Individual and mean($\pm$ SEM) white blood cell, total lymphocyte, and platelet counts in 7 healthy men during the regular period

Subject	White blood cell	Total lymphocyte	Platelet
		$\frac{9}{10} / L$	
1	11.5	3.80	240
2	7.5	2.83	154
3	6.5	3.06	200
4	6.6	2.61	253
5	10.0	4.40	226
6	4.5	1.40	188
7	6.5	1.78	216
Mean $\pm$ SEM	7.6 $\pm$ 0.9	2.84 $\pm$ 0.40	211 $\pm$ 13

Table 51. Individual and mean($\pm$ SEM) white blood cell, total lymphocyte, and platelet counts in 7 healthy men during the ovovegetarian period

Subject	White blood cell	Total lymphocyte	Platelet
		⁹ 10 /L	
1	10.6	2.23	256
2	6.1	2.30	225
3	7.5	2.69	230
4	5.2	2.06	250
5	8.9	3.65	244
6	5.9	2.24	270
7	6.6	1.78	240
Mean $\pm$ SEM	7.3 $\pm$ 0.7	2.42 $\pm$ 0.23	245 $\pm$ 6

Dietary protein is quantitatively used for depositing new protein in tissues of pregnant women, infants and children, for the protein secretion in milk of lactating women, and for the maintenance of body protein synthesis in adults. Thus inadequate protein intake causes diminished protein content in cells and organs and deterioration in the cellular capacity to perform their normal function. This leads to increased morbidity and mortality. On the other hand, excess protein intake of physiologic need is also disadvantageous (1). Therefore an adequate diet must contain an appropriate level of protein for the assurance of long-term health.

Safe levels of protein intake according to sex and age groups have been recently proposed by FAO/WHO/UNU (23). The greatest need of protein in proportion to body size is infants (1.85-1.50 g/kg/day for 3-12 mo) and young children (1.20-1.10 g/kg/day for 1-5 yr). As individuals proceed to childhood the requirement diminished gradually (1-0.95 g/kg/day for 5-14 yr; 0.94-0.80 g/kg/day for 14-18 yr) until the static requirement of adulthood is reached (0.75 g/kg/day). Additional protein intake should be provided during pregnancy (+6 g/day) and lactation (+17.5-13 g/day). However, these numbers apply to highly digestible proteins such as those of eggs, milk, meat, or fish.

It is usually accepted that the relative concentration of indispensable amino acids is the major factor

determining the nutritional value of food protein. Most animal proteins have a satisfactory indispensable amino acid pattern in relation to the amino acid requirements. Therefore animal proteins are of high quality. On the other hand vegetable protein may be of lower nutritional value because they tend to be limiting in one or more of the indispensable amino acids.

Lysine is the first limiting amino acid in rice, maize, and wheat whereas that in soybean is methionine (10). The limiting amino acid may lead to poor utilization of amino acids by humans and relatively more of the protein is required to meet the nutritional requirement. Such deficiencies can be corrected either by adding the limiting amino acids or complementation with other protein sources.

Legumes and seeds such as soybean, mungbean, groundnut, sesame, and cottonseed have higher protein content than cereals (10). They have been employed to improve the nutritional value of cereal-based diets which are the main cause of PCM.

The pioneer work of Scrimshaw et al (25) has shown that Incaparina, a vegetable mixture consisting of 29% whole ground corn, 29% whole ground sorghum, 38% cottonseed flour, 3% Torula yeast, 1% calcium carbonate, and 4,500 IU of vitamin A per 100 g, is effective protein sources for the treatment of kwashiorkor. When compared

with isonitrogenous amounts of milk in alternate 5-day nitrogen balance periods in young children at adequate levels of intake, the amount of nitrogen retained as percent of intake was not significantly different. Subsequent reports by various investigators who studied the nitrogen retention in children receiving soy-cereal diets, expressed in relation to nitrogen retention with milk, have shown the nutritional value of soy in combination with rice or corn flour or with mixed sources such as milk solids and corn meal closely approaching that of milk (26).

To alleviate the problem of PCM in Thai preschool children, the Division of Nutrition, Ministry of Public Health has implemented the production and dissemination of supplementary food mixtures in more than 10,000 rural villagers by community participation. Among these supplementary food mixtures are rice: soybean:groundnut (70:15:15), rice:soybean:sesame (70:15:15), rice:mungbean:groundnut (60:15:20), and rice:mungbean:sesame (60:20:15). The energy contents in these mixtures are 437, 448, 443, and 451 kcal/100 g, respectively, whereas the respective protein contents are 16.5, 14.8, 14.5, and 13.2 g/100 g. The formulation of these mixtures is based on the Thai regulation for infant foods stating that the protein content should not be less than 2.5 g/100 available kcal, the protein quality expressed in term of amino-acid score

should not be less than 70% of the FAO/WHO reference pattern, the fat content should not be less than 2 g and should not be more than 6 g/100 available kcal, and linoleate content should not be less than 300 mg/100 available kcal. As a consequence of this implementation, there is a decline in the prevalence of PCM by weight for age in these preschool children from 51% in 1982 to 24% in 1986 (27).

In 1983, there was an outbreak of beriberi in institutionalized adolescents at the Observation Protection Center (OPC) of Rayong Province, eastern Thailand (28). Dietary factors causing inadequate thiamin intake in these institutionalized adolescents include high consumption of milled rice, loss of thiamin due to methods of cooking, and lack of consumption of rich sources of thiamin. Dietary assessment revealed that 10, 11, and 79% of their total energy intake before supplementation (wk 0) were derived from protein, fat, and carbohydrate, respectively, and 65% of their protein intake came from vegetable sources, mainly rice (Table 52).

Due to the constraint of food budget at OPC we had introduced the soybean supplementation program in these adolescents to improve their nutritional status. Soybean is chosen because it is locally available, relatively inexpensive, and highly nutritious. The soybean supplementation included 164 males and 19 females, aged 10-19

Table 52. Mean energy intake and its distribution in institutionalized adolescents

Nutrient	Wk 0		Wk 4		Wk 8	
	Amount	%	Amount	%	Amount	%
Energy (kcal)	2,627	100	3,048	100	3,116	100
Protein (g)	66	10	74	10	84	11
Animal (g)	23	3.5	19	2.6	28	3.3
Vegetable (g)	43	6.5	55	7.4	56	6.7
Fat (g)	31	11	51	15	40	12
Carbohydrate (g)	510	79	562	75	587	77

Table 52. Mean energy intake and its distribution in institutionalized adolescents

Nutrient	Wk 0		Wk 4		Wk 8	
	Amount	%	Amount	%	Amount	%
Energy (kcal)	2,627	100	3,048	100	3,116	100
Protein (g)	66	10	74	10	84	11
Animal (g)	23	3.5	19	2.6	28	3.3
Vegetable (g)	43	6.5	55	7.4	56	6.7
Fat (g)	31	11	51	15	40	12
Carbohydrate (g)	510	79	562	75	587	77

yr. Each subject took 200 ml of soybean milk which was prepared freshly in the morning everyday, except Saturday and Sunday. The soybean residue left after the preparation of soybean milk was used to mix with animal protein to be served at lunch and dinner. Each 100 g of soybean milk provided 48 kcal, 2 g protein, 0.7 g fat, and 8 g carbohydrate whereas each 100 g of soybean residue provided 75.8 kcal, 6 g protein, 2.8 g fat, and 7 g carbohydrate.

Dietary assessment at wk 4 and 8 after soybean supplementation revealed the increase in total energy and protein intakes in these adolescents (Table 52). Soybean milk and residue constituted 3-4% of their energy intake and 5-7% of their protein intake during the supplementation. Table 53 shows that their serum total protein and albumin levels at wk 4 and 8 and nitrogen balance at wk 8 were significantly higher than those at wk 0. These data indicate the improvement of protein status in these adolescents by soybean supplementation.

Since soybean provides high protein content with low cost compared with meat protein. The present study had been carried out to investigate the beneficial effects of soybean as the major dietary protein source on protein-calorie status in healthy men. Besides, we had employed natural soybean foods as the protein source (Table 3). Our study is different from other using processed soybean,

Table 53. Effect of soybean supplementation on serum transport protein and nitrogen balance levels in institutionalized adolescents

Parameter	Mean±SEM		
	Wk 0	Wk 4	Wk 8
Serum total protein (g/L)	71.3±0.5	78.3±0.4	78.0±0.3 ^a
Serum albumin (g/L)	39.1±0.3	43.9±0.2 ^a	44.5±0.2 ^a
Nitrogen balance (g/day)	5.51±0.19	5.47±0.16	7.05±0.14 ^{a,b}
^a Wk 4, Wk 8 > Wk 0: P<0.001, ^b Wk 8 > Wk 4: P<0.001			

i.e., soy protein isolates or soy protein concentrates (29).

Diet plays the important role in the prevention and treatment of hypercholesterolemia. Regarding to energy and fat intake, the following recommendations to lower serum cholesterol levels have been made for the general population and for high risk individuals: control of obesity by decreasing energy intake and by exercise, reduction of the intake of saturated fatty acids to less than 10% of total dietary energy, increase the use of oleic and linoleic acids to 10% of total dietary energy for each fatty acid, and reduction of dietary cholesterol to less than 300 mg/day (30,31). Thus total fat intake was assigned to be 30% of total dietary energy (Table 7) and soybean oil was used to cook foods during the VD and OV periods because soybean oil is the excellent source of linoleic and alpha-linolenic acids, the essential fatty acids for man. Our analysis revealed that the soybean oil preparation used in this study had the following fatty acid composition expressed as percentages of total fatty acids: 53% of linoleic acid (18:2 n-6), 7% of alpha-linolenic acid (18:3 n-3), 0.1% of palmitoleic acid (16:1 n-7), 26.5% of oleic acid (18:1 n-9), 0.2% of eicosenoic acid (20:1 n-9), 0.2% of myristic acid (14:0), 9.2% of palmitic acid (16:0), 3.4% of stearic acid (18:0), and 0.1% of arachidic acid (20:0). During the VD and OV

periods, soybean oil was used at 20% of total dietary energy which, in turn, provided 10.6% of total dietary energy as linoleic acid, 1.4% as alpha-linolenic acid, 5.4% as monounsaturated fatty acids (16:1 n-7, 18:1 n-9, and 20:1 n-9), and 2.6% as saturated fatty acids. The VD did not contain cholesterol whereas the mean ($\pm$ SEM) cholesterol intake in these subjects during OV was 556 ± 20 mg/day.

Regarding to protein and carbohydrate intake, they are recommended to be 10-20% and 50-60% of total calories (30,31). In the present study, they were 15 and 55% of total dietary energy (Table 7).

Energy intake for each subject receiving PF, (Table 6) VD, or OV (Table 7) was isocaloric based on his mean energy intake plus 1 SD observed during the 14 days of the BL period (Table 5). Their energy intake was increased from the BL period in order to reassure the adequate energy intake.

In the present study, both the VD and OV periods were preceded by a one-day PF period in order to reduce the necessary length of the adaptation period when the test protein is fed either at or below requirement level (32). It should also be noted that during the PF period the mean protein, fat, and carbohydrate intake were 0.2, 30, and 69.8% of total dietary energy, respectively (Table 6).

During the VD and OV periods, the subjects consumed all of the meals served were satiated, and satisfactory with food menus.

This was evidenced by higher energy intake during these periods than the RD period (Table 9). However, their energy intake during the PF, VD, OV, and RD periods were lower than the estimated energy requirement (Tables 9 and 10). This may explain why their mean body weight and BMI decreased significantly during the study (Tables 17 and 18). The decrease in their body weight was mainly due to the loss of adipose tissue. This was supported by the significant decrease in body fat (Tables 19 and 20) and waist and hip circumferences (Tables 26 and 27). There was no significant change in waist/hip ratio during the study (Table 28) because of the simultaneous decreases in waist and hip circumferences (Tables 26 and 27).

In the present study, the protein intake in all of the subjects was at 15% of total dietary energy. Thus their mean protein intake during VD and OV was isonitrogenous, i.e., 1.18 g/kg/day (Tables 11 and 12) which was higher than the calculated safe levels of protein intake of 0.90 g/kg/day for VD and 0.87 g/kg/day for OV (23).

It is well established that the needs for protein and for each IDAA decline progressively with increasing age from infancy. The estimated requirements of total IDAA, excluding histidine, are 680 mg/kg/day for infants,

352 mg/kg/day for preschool children (2-5 yr), 216 mg/kg/day for school children (10-12 yr), and 187 mg/kg/day for adults (23,24). The IDAA requirement for adults is based on the newly revised estimates. The previous IDAA requirement recommended by FAO/WHO/UNU is 84 mg/kg/day for adults (23).

It has long been believed that the requirements for IDAA decrease more extensively than do those for total protein. The safe levels of protein intake should constitute of 43% of IDAA for infants, 32% for preschool children, 22% for school children, and 11% for adults (23). However, the new adult pattern of IDAA requirement is quite similar to that proposed by FAO/WHO/UNU for the preschool children, except for a lower threonine (25 vs 34 mg/g.protein) and slightly lower lysine 50 vs 58 mg/g.protein content in the newly proposed adult pattern (23,24). Thus the nutritional value of the different protein sources would not be affected as markedly by the age of the subjects. Based on this knowledge, it follows that for an evaluation of dietary protein quality, it is probably only necessary to use two amino acid requirements or scoring patterns; one for infant [which is based on the amino acid composition of breast milk (23)] and the other as shown in (Table 13), for all groups above about 2 yr of age (24). In our study, the IDAA intakes during the VD and OV periods were higher than the newly revised

estimated of IDAA requirement for adults (Tables 13 and 14). Besides, the mean IDAA intake during the VD and OV periods was 38.6 and 39.8% of total amino acids (Tables 13-16). Thus the protein sources of VD and OV are quantitatively and qualitatively adequate.

Clinical methods for the evaluation of protein quality are based on the same principles applied to the corresponding animal assays but require some modification for application to human subjects. The major procedures and criteria used are growth, (e.g., weight, height, creatinine-height index), blood and serum constituents, (e.g., serum transport proteins, enzymes, free amino acids, urea nitrogen), and metabolic balance, (e.g., nitrogen excretion and sulfur excretion). Growth or nitrogen balance may be used alone or in combination with biochemical analysis of serum constituents. The majority of studies conducted in humans for determining the nutritional value of soybean protein have been based on measures of growth, nitrogen balance and nitrogen utilization in infants and children and on indices of nitrogen balance in adolescents and adults (26).

The nitrogen-balance technique involves the determination of the difference between the intake of nitrogen and the amount excreted in urine, feces, and sweat, together with minor losses by other routes. In most experiments only the nitrogen contents of the diet, urine,

and feces have been directly measured. Allowances are made for losses by other routes on the basis of a limited number of published studies. One problem encountered in nitrogen balance technique is the magnitude of the nitrogen losses other than from urine and feces. The most important of which is via the skin. During heavy work in hot climates appreciable amounts of nitrogen are lost in sweat, mainly as urea, although the losses are lower in those who are acclimatized (33). There is some evidence of a compensatory reduction in urinary urea output (34). The nitrogen content of sweat is related to blood urea and both decrease with a low protein intake. In the conventional balance study, the losses are not determined and an estimated allowance is made for them. On the basis of the available evidence this allowance has been set at 8 mg of nitrogen/kg/day for adults and 10 mg/kg/day for children up to the age of 12 yr. It is unlikely that a singly figure will be applicable under all conditions, but there is no realistic alternative to using this method of correction (23).

A further important consideration is the length of time needed to achieve a steady state at given levels of protein intake. With abrupt change from a normal protein intake to a nitrogen-free diet, 5 to 7 days are required for stabilization of urinary nitrogen excretion in young adults, and 4 to 5 days in young children (32).

Our study has employed the aforementioned principle for the nitrogen-balance study. After a one-day PF period, the subjects consumed the VD for 10 days. The first 5 days for their adaptation. Nitrogen balance was measured for the last 5 days. To avoid the carrying-on effect of the VD, the subjects were allowed to consume the RD ad libitum for 3 days followed by a one-day PF prior to the OV period lasting for 10 days. Nitrogen balance was then measured for the last 5 days of the OV period.

Studies on the utilization of dietary protein by human adults have been directed primarily for the establishment of minimal requirements (35). Limited data are available for the dietary protein that exceed minimal requirements but are consumed by many adults. Our study was not designed to estimate for protein and amino acid requirements. However, nitrogen balance was employed to assess the adequacy of protein intake which was assigned at 15% of total dietary energy usually consumed by the subjects. During the VD period, their mean energy intake was 31.7 kcal/kg/day (**Table 10**) while their mean nitrogen intake was 12.15 g/kg/day (**Table 42**). All of the subjects exhibited positive nitrogen balance with a mean of 36.42 mg/kg/day. This indicates that soybean can be used as a major dietary protein source for adults at 15% of their total dietary energy.

Soybean like other legumes contains a wide variety of biologically active substances, e.g., trypsin inhibitor, hemagglutinin, and saponin. These antinutritional factors inhibit the digestibility, the absorption, and the utilization of the protein of raw soybean. Most of these factors can be partially or wholly inactivated by heat treatment. The protein digestibility of boiled soybean is 92% whereas that of roasted soybean flour is only 78%. Processing of soybeans by means of fermentation, coagulation or isolation also improves the digestibility. The protein digestibility of soy protein isolate, soybean curd, deep fried soybean curd, light fried soybean curd, congealed soybean curd, yoba, and fermented soybean are 100, 97, 91, 97, 93, 100 and 90%, respectively (36).

Besides nitrogen balance, the quality of dietary protein can be evaluated by several methods including IDAA content, apparent digestibility $[(\text{nitrogen intake} - \text{fecal nitrogen}) / \text{nitrogen intake}]$, apparent biological value $[(\text{nitrogen intake} - \text{fecal nitrogen} - \text{urinary nitrogen}) / (\text{nitrogen intake} - \text{fecal nitrogen})]$, and apparent net protein utilization $[(\text{nitrogen intake} - \text{fecal nitrogen} - \text{urinary nitrogen}) / \text{nitrogen intake}]$ (32). In our study, the mean apparent digestibility was 84.9% for VD and 85.8% for OV. The mean apparent biological value was 26.9% for VD and 25.9% for OV. The mean apparent net protein utilization was 22.8% for VD and 22.2% for OV (Tables 42 and 43). As

already pointed out that the efficiency for the utilization of specific dietary proteins or their combination depends not only on their quantity but also their quality which is determined by the constituent of IDAA. For instance, the total amount of cereal proteins to fulfill minimal protein requirement of adults can be reduced markedly by combining foods that differ in amino acid composition but complement each other (36). In our study, when 20% of soybean protein was replaced by egg protein during the OV period (Tables 11 and 12) the subjects had higher IDAA intake during the OV period than the VD period (Tables 13 and 14). However, their nitrogen balances between these two dietary periods were not significantly different (Tables 42 and 43). This indicates that partial substitution of soybean protein by egg protein while total nitrogen and energy intake being kept constant no further increase in positive nitrogen balance was observed. This is consistent with the similar figures of apparent digestibility, biological value, and net protein utilization of VD and OV as already mentioned.

Previous study by Clark et al (37) has also shown the effect of protein quantity and quality on nitrogen balance and digestibility. When 7 young men, aged 22-28 yr, consumed diets containing 16, 14, 12, 10 and 8 g of nitrogen per day of which rice, wheat, chicken, and milk provided 25, 15, 30, and 30%, respectively, the corres-

ponding mean apparent nitrogen balances were 2.92, 2.01, 1.46, 0.90, and 0.48 g/day. During the 12 g nitrogen period the mean daily energy and nitrogen intake were 44.6 kcal/kg and 165 mg/kg. In our study, during the VD and OV periods supplying 12.1 g nitrogen/day the mean daily energy and nitrogen intake were 31.7 kcal/kg and 188.8 mg/kg (Tables 10 - 12). The mean daily nitrogen balance in our subjects was 36.42 mg/kg whereas that in Clark et al (37) was 12.08 mg/kg. On the contrary, the apparent digestibility of dietary protein in the Clark's study was 89.8% which was higher than our study, 84.9% for VD and 85.8% for OV.

Our data indicate that despite of insufficient energy intake, nitrogen balance of the subjects could be maintained if their nitrogen intake was adequate. This is also supported by the anthropometric data which revealed the preservation of the somatic protein status, i.e., no significant loss in body muscle mass (Table 22) upper arm muscle circumference (Table 24) and bone-free arm muscle area (Table 25).

Serum proteins provide a more sensitive index of protein status than is reflected by anthropometric data. Virtually all serum proteins decrease as a consequence of PCM. The extent to which different serum proteins decrease is largely a function of the half-life of the protein measured. Knowledge of the half-life of various

serum proteins allows interpretation of their absolute value to be usefully employed. Besides serum protein levels may be influenced by factors other than protein intake. In general, the rate of total body protein synthesis, breakdown, and turnover decreases in patients with PCM and returns to normal in parallel with nutritional recovery. Visceral protein analysis includes measurement of serum albumin, transferrin, retinol-binding protein, and thyroxine-binding prealbumin (38). In our study only the first three serum proteins and total protein were measured.

Albumin, from week 20 of gestation and throughout life, is the most abundant protein in human serum, representing 55-65% of the total protein. It is synthesized in the liver at a rate that is dependent on protein intake but subject to feedback regulation by the serum albumin level. The molecule contains no carbohydrate and is not stored to any extent in parenchymal cells. Little albumin is filtered through the renal glomeruli, and most of that is reabsorbed by the proximal tubule cells and degraded by their lysosomal enzymes into fragments that are returned to the circulation (20).

Albumin has a molecular weight of approximately 66,000. The exchangeable albumin pool is about 4 g/kg in women and 10-20% greater in men. Normally only one-third of the total exchangeable albumin resides in the intravas-

cular space whereas the remaining is distributed through the extravascular space in the skin, muscle, and viscera. Fifteen grams of albumin are synthesized and catabolized daily, and newly produced albumin has a half-life of about 17 days (20,38).

The chief biological functions are to transport and store a wide variety of ligands, to maintain the plasma oncotic pressure, and to serve as a source of endogenous amino acids. Hypoalbuminemia is very common in many illnesses resulting from one or more of the following factors: inadequate protein intake, protein malabsorption, impaired biosynthesis, altered distribution that may sequester large amount of albumin in an extravascular compartment, protein loss via urine or feces, and increased protein catabolism (20).

Serum total protein and albumin levels have been used for the longest time in assessing the human protein-calorie status. Serum total protein appear to be of little value as a sensitive index for estimating protein intake or protein nutritional status. Subjects with inadequate protein-calorie status and having hypoalbuminemia may have elevated serum total protein level resulting from an increase in the alpha-globulin fraction, frequently in association with infection and parasitic infestation. In adults, serum total protein levels of less than 60, 60-64, and 65 g/L and above are considered

to be deficient, low, and acceptable protein status, respectively, whereas the corresponding figures for serum albumin levels are less than 28, 28-34, and 35 g/L and above (39). In most series, serum albumin level of less than 30 g/L correlate indirectly with morbidity and mortality. In our study, all of the subjects had serum total protein and albumin concentrations in the acceptable levels. Besides there was no striking change in these two parameters during the study (Table 30).

Since serum albumin level is not highly specific or rapid indicator of change in protein-calorie status most recent studies have included the determination serum transferrin and RBP.

Transferrin is synthesized in the liver and to a small extent in the reticuloendothelial system and in endocrine glands such as testes and ovaries. It is a glycoprotein with a single polypeptide chain and is a principal serum protein for transport of iron. It reversibly binds numerous polycations, namely, iron, copper, zinc, cobalt, and calcium, although only iron and copper binding appear to have physiological significance. One molecule of transferrin binds two ferric ions and an associated anion that is, in vivo, usually bicarbonate. There are two binding sites for iron, probably with different affinities, they are strong at physiological pH but weaken with decreasing pH (38).

Serum transferrin has a half-life of 8 days. Its level is apparently regulated by the availability of amino acids and iron. Metabolic studies done on obese women given various protein diets supplemented with high or low caloric intake showed decreasing levels of transferrin when protein intake was low. Significant decreases occurred, however, only when calories were also restricted, as severe energy deprivation, with adequate protein intake, may be accompanied by relatively normal transferrin values. In many primary disease states associated with coexisting iron deficiency, anemia may stimulate transferrin production, even in the presence of PCM, and may accentuate any putative correlation between PCM and transferrin values (38).

Serum transferrin level of less than 200 mg/dL is used to diagnose inadequate protein status (40). Our subjects had inadequate total energy intake with sufficient protein intake during consuming various diets. This may explain why their mean serum transferrin levels during the study tended to be lower than that during the BL period but these changes did not reach statistical significance (Table 31). Only the mean serum transferrin level at the VD period was lower than 200 mg/dL.

Retinol-binding protein is the serum protein with a shorter half-life and more biologic specificity than albumin, or transferrin. Vitamin A is mobilized from the

hepatic stores as an unesterified retinol, bound to the specific carrier, RBP. RBP has a molecular weight of 21,000 and has a single binding site for one molecule of retinol. RBP circulates normally bound in a protein complex the thyroxine-binding prealbumin having a molecular weight of 55,000. The interaction between these two proteins is specific, and the affinity is high (38).

RBP is synthesized by the liver. Its synthesis is very much dependent upon the nutritional status of the patient, and is very much impaired in PCM and in patients with chronic liver disease. Its synthesis requires zinc, which seems to be important for vitamin A mobilization. The RBP is present as free form in small amounts in the normal plasma and is in equilibrium with the prealbumin RBP complex. Plasma concentration of free RBP is about 5 mg/L and the concentration of the bound RBP is 45 mg/L. The biologic half-life of the free RBP is about 4 hr, and the bound RBP, 11 hr. Being a low molecular-weight protein, the RBP passes easily through the glomerular barrier, and is subsequently reabsorbed and catabolized by the cells of the proximal tubuli.

Previous study in 12 healthy but obese patients on starvation diets showed much more acute changes in serum RBP than serum transferrin levels. Serum RBP level appeared to develop a rapid decrease, levelling to a plateau at one week, whereas serum albumin and transferrin

levels gradually decreased. It has also been observed that RBP decreased when deficits of either protein or calories were administered. In addition, like albumin, RBP is depressed with severe liver disease. Other chronic disorders, such as hyperthyroidism and vitamin A deficiency, may cause low serum level of RBP and reflect specific diseases, not just PCM (38).

In our laboratory, low serum RBP is diagnosed when its level less than 35 mg/L. Throughout the study, the mean serum RBP levels in our subjects were above 35 mg/L. However, their RBP levels at the VD, RD, and OV periods were significantly lower than that at the BL period (Table 31). Since their protein intake during the study periods exceeded the safe level. The decrease in RBP levels could be due to inadequate energy intake (Table 9) which was supported by the decrease in body weight, BMI, and body fat (Tables 17-19). However, the decrease in RBP due to low vitamin A intake should also be considered because their vitamin A intake during the VD period was derived from corotene only whereas the major source of their vitamin A intake during the OV period also came from carotene.

Urea is the major nitrogen-containing metabolic product of protein catabolism in humans, composing over 75% of the nonprotein nitrogen eventually excreted. The biosynthesis of urea from amino nitrogen-derived ammonia is carried out exclusively by hepatic enzymes of the urea cycle. Over 90% of urea is excreted through the kidney.

The determination of serum urea nitrogen has been commonly employed for the assessment of renal function. The normal range for serum urea nitrogen level of healthy ambulatory adults is 7-18 mg/dL. A wide variety of renal diseases can cause an increase in serum urea nitrogen. However, elevated serum urea nitrogen level can also be caused by non-renal factors which include mild dehydration, high protein diet, increased protein catabolism, reabsorption of blood proteins after a gastrointestinal hemorrhage, treatment with cortisol or its synthetic analog, or decreased renal perfusion (20). Our subjects exhibited normal serum urea nitrogen levels throughout the study (Table 29). This implies their normal renal function. The significantly higher serum urea nitrogen concentration at the VD and RD periods than the BL period was most likely due to their high protein intake (Tables 11, 12 and 29).

Throughout the study plasma glucose levels of our subjects were within normal limit (Table 29). The significantly lower plasma glucose level at the RD period than the BL period was most likely due to the lowest energy intake during the RD period (Table 9).

The renal glomeruli behave as ultrafilters for serum proteins. The degree to which individual proteins are normally filtered through the membrane is dependent on both their molecular size and their serum concentration.

In general, transport of protein molecules through the glomerular membrane progressively diminishes as protein size increases. Normally, high-molecular-weight protein such as IgM having a molecular weight of 900,000 do not appear in glomerular filtrate except in trace amounts. Relatively small yet significant amounts of albumin having a molecular weight of 66,000 are passed into the filtrate as a result of its high plasma concentration and relatively low molecular weight. Proteins with a molecular weight of 15,000-40,000 filter more readily but in lesser quantities because of their low serum concentrations. The proportions of individual proteins excreted in the urine depend on the extent of their reabsorption by the renal tubules. The lower-molecular-weight proteins, including some albumin, are actively reabsorbed from the filtrate and catabolized by the proximal tubules. Consequently, the total protein excreted is only 20-150 mg/day; about half is albumin and the remainder almost entirely Tamm-Horsfall protein (uromucoid), a constituent of urinary casts and probably secreted by the distal tubules (20).

Urinary protein excretions in our subjects throughout the study were within the normal limit (Table 37). Our study has shown the influence of protein intake on urinary protein excretion. During the PF period they had the lowest urinary protein excretion which was also significantly lower than those during the VD, RD, and OV

periods. The lack of significant differences in urinary protein excretion between the BL and the other periods was due to the greater wide variation of urinary protein excretion in each subject during the BL period than the other periods (**Tables 32-36**).

In 1974, Huse et al (41) reported the use of the 24 hr urinary nitrogen excretion as a validity test for dietary nitrogen intake. In his study, diet interview data obtained from patients with coronary heart disease were not reliable; only 16 of 60 patients gave accurate diet histories; there was no significant relationship between their dietary nitrogen levels and urinary nitrogen excretion. After 4 days in the Clinical Research Center, however, a highly significant correlation was found between dietary nitrogen intake and urinary nitrogen excretion in these 60 patients ($y=1.757+0.896x$, $r=0.60$, $p<0.01$). In 1980, Isaksson (42) demonstrated that nitrogen intake estimated from 24 hr urinary nitrogen excretion agreed with nitrogen intake derived from dietary history or dietary record whereas there was a systematic underestimation using the 24-hr recall method. In 1985, Bingham and Cummings (43) also verified urinary nitrogen excretion as an independent validating dietary method of measuring nitrogen intake in individuals. The authors had measured nitrogen intake and excretion in 8 healthy subjects on their usual, varying, diets for 28 days. The mean daily

energy intake of these subjects ranged from 2,020 to 4,340 kcal with an average of 2,930 kcal. Their mean daily nitrogen intake ranged from 11.6 to 21.3 g with an average of 15.9 g whereas their mean daily urinary nitrogen excretion ranged from 9.67 to 17.16 g with an average of 12.85 g. The within person variation in nitrogen intake ranged from 14 to 26% (coefficient variation, CV) while urinary nitrogen was more constant varying from 11 to 18% (CV) within individuals.

Our study has also demonstrated that urinary nitrogen excretion reflects the amount of nitrogen intake. This is supported by the following findings: (a) the highest urinary nitrogen excretion was observed during the RD period, the highest protein intake period, whereas the lowest urinary nitrogen excretion was observed during the PF period (**Tables 5-8 and 37**), (b) during isocaloric and isonitrogenous intake of the VD and OV periods, there was no significant difference in urinary nitrogen excretion (**Tables 7 and 37**), and (c) there was significant positive correlation between nitrogen intake and urinary nitrogen excretion in g/day ($y=5.082+0.197x$, $r=0.518$, $df=33$, $t=3.4803$, $P<0.005$).

During the last 3 days of BL period within person variation in nitrogen intake ranged from 4 to 55% (CV) while urinary nitrogen excretion varied from 6 to 45% (CV) (**Table 38**). During the 3 days of the RD period, the

nitrogen intake in each individual was constant whereas their urinary nitrogen excretion ranged from 5 to 42% (Table 40). During the 10 days of VD and OV periods nitrogen intake in each individual was also held constant there was still variation in urinary nitrogen excretion within individuals, i.e., 10-39% for VD (Table 39) and 13-54% for OV (Table 41).

Bingham and Cummings (43) reported that in 8 subjects with the mean daily nitrogen intake of 15.9 g their mean daily urinary nitrogen excretion was 12.85 g which was 81% of other nitrogen intake.

We previously found that in 12 subjects on their regular diets providing the mean daily nitrogen intake of 15.6 g their mean daily urinary nitrogen excretion was 10.7 g which was 68% of their nitrogen intake (44). In the present study, the mean daily nitrogen intake in the 7 subjects on VD was 12.14 g whereas their mean daily urinary nitrogen excretion was 7.54 g which was 62% of nitrogen intake (Table 42). The corresponding figures for OV were 12.14 g, 7.72 g, and 64% (Table 43).

In 1979, Cummings et al (47,48) reported the influence of the amount of dietary fiber intake on fecal weight and fecal nitrogen in four subjects who were fed 3 types of diets, i.e., low protein diet, high protein diet, and high protein diet with added wheat fiber. Low protein diet provided 2,880 kcal/day, 10.1 g nitrogen, and 23.4 g

dietary fiber. High protein diet provided 2,880 kcal/day, 21.9 g nitrogen, and 21.8 g dietary fiber. High protein diet with added wheat fiber provided 2,772 kcal/day, 26.9 g nitrogen, and 53.2 g dietary fiber (31 g from wheat fiber). They consumed each diet for 3 weeks. The mean ($\pm$ SEM) fecal nitrogen excretion during the low and high protein diets were 1.26 ± 0.2 and 1.40 ± 0.3 g/day, respectively, which were not significantly different. However, adding 31 g dietary fiber per day from wheat to the high protein diet their fecal nitrogen excretion rose to 2.5 ± 0.1 g/day which was significantly higher than those during the low and high protein diets. The mean ($\pm$ SEM) fecal weight during high protein diet with added wheat fiber was 210 ± 9 g/day which was significantly higher than 80 ± 11 g/day during high protein diet and 80 ± 10 g/day during low protein diet. They concluded that the increase in fecal weight and fecal nitrogen excretion was due to a greater dietary protein entry to the large bowel affected by the amount of dietary fiber with the subsequent increased bacterial cell population.

Our Previous study (44) in 12 healthy subjects fed diets providing a daily supply of 2,265 kcal and 15.6 g nitrogen mainly derived from pork (pork diet) for 5 days revealed the mean $\pm$ SEM fecal weight of 108 ± 6.8 g/day and fecal nitrogen of 1.24 ± 0.14 g/day. In the present study, both VD and OV provided 1,995 kcal and 12.14 g nitrogen.

During receiving VD and OV, the mean($\pm$ SEM) fecal weight of the 7 subjects were 184 ± 29 and 142 ± 24 g/day whereas the corresponding figures for their fecal nitrogen excretion were 1.8 ± 0.11 and 1.7 ± 0.32 g/day. These data indicate that though nitrogen intake from VD and OV was lower than pork diet opposite results were observed for the fecal weight and nitrogen. Statistically significant differences in these parameters were also observed between VD and pork diet ($P<0.05$ for fecal weight, $P<0.005$ for fecal nitrogen). These differences were most likely due to increased dietary fiber intake derived from VD and OV and consistent with the suggestion of Cummings et al (47,48). It should be noted that dietary fiber intake in our subjects was not estimated because of unavailability of food composition table for dietary fiber content present in the diets employed in our study.

Regarding to hematological status, the subjects were not anemic throughout the study evidenced by normal hemoglobin, hematocrit, red blood cell count, MCV, MCH, and MCHC (**Tables 44-47**) (49). Their white blood cell, total lymphocyte, and platelet counts during the study were also within normal limits (**Tables 48-51**). Their mean total lymphocyte count greater than 1.5×10^9 /L indicated the competency of their cell-mediated immune response (39).

CHAPTER IV

INFLUENCE OF SOYBEAN COMPARED WITH SOYBEAN-EGG DIETS
ON CREATININE METABOLISM

Results

Water intake

Tables 54-56 show mean and range of water intake in ml/kg/day as well as its CV and range expressed as the percentage of mean in 7 healthy individuals during the VD, RD, and OV periods. Water intake during the last 3 days of the BL period was not recorded. Table 57 shows water intake in these subjects at days 1 and 15 which were the PF periods.

Table 58 shows mean($\pm$ SEM) water intake in 7 healthy men on various diets, which were computed using the same principle as described in mean urinary protein excretions Table 37. During the PF period, the mean($\pm$ SEM) water intake was significantly lower than those during the VD, RD, and OV periods. The mean($\pm$ SEM) water intake at the RD period was significantly higher than that at the OV period (Table 58).

Urinary volume

Mean urinary volume in ml/kg/day, its CV, and range expressed as the percentage of mean in our 7 subjects during the VD, RD, and OV periods are shown in Tables 59-62. Table 57 shows urinary volume in these subjects

during the PF period.

Table 58 shows mean($\pm$ SEM) urinary volume in the 7 subjects on various diets, which were computed using the same principle as described in mean urinary protein excretion (**Table 37**). The mean($\pm$ SEM) urinary volume in ml/kg/day at the BL period was significantly lower than those at the VD, RD, and OV periods. Similar findings were observed when urinary volumes were expressed in ml/day between the BL and RD or OV periods.

Urinary creatinine

The constancy in the urinary creatinine excretions were evaluated in our subjects throughout the study. The mean urinary creatinine in mg/kg/day, its CV, range in mg/kg/day and percentage of mean during the BL, VD, RD, and OV periods are shown in **Tables 63-66**. Subject 7 had the greatest CV during the BL, VD, and OV periods (**Tables 63, 64, and 66**). During the PF period, days 1 and 15, the individual and mean($\pm$ SEM) urinary creatinine excretions expressed in mg/kg/day are shown in **Table 67**.

Using the same principle of calculation as described in mean urinary protein excretion (**Table 37**), **Table 68** shows the mean($\pm$ SEM) urinary creatinine excretion of 7 subjects during the various diets. There were no statistically significant differences in urinary creatinine excretions among various diets.

Serum creatinine and creatinine clearance

Table 69 shows individual and mean($\pm$ SEM) serum creatinine in 7 healthy men on various diets. Throughout the study their serum creatinine levels were within the normal limit (47). The mean($\pm$ SEM) serum creatinine level at the OV period, was the lowest and significantly different from that at the VD period only.

Body surface area of each subject was calculated, using DuBois' formula: $S = 71.84 W^{0.425} H^{0.725}$ where S equals surface area in square meters, W equals body weight in kg, and H equals height in meters (48). Table 70 shows body surface area in each individual, mean surface area of the 7 subjects and the percentages of these values compared with 1.73 m^2 , the reference of body surface area. Their mean($\pm$ SEM) body surface area during the VD, RD, and OV periods were significantly lower than that during the BL period whereas this parameter in m^2 during the VD and RD periods were significantly higher than that during the OV period. Their body surface area in percentage of 1.73 m^2 during the OV period was significantly higher than that during the VD period.

Table 71 shows individual and mean($\pm$ SEM) creatinine clearance in 7 healthy men on various diets expressed as ml/min and ml/min/ 1.73 m^2 . There were no statistically significant differences in creatinine clearance throughout the study.

Table 54. Water intake in 7 healthy individuals on vegetarian diet (10 days)

Subject	Water intake			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	40.2	31	28.7-61.0	71-152
2	25.7	24	16.3-37.6	71-164
3	23.5	18	19.3-32.2	109-182
4	22.1	20	16.6-30.5	75-138
5	20.2	29	12.5-29.7	62-147
6	29.9	31	16.1-47.6	54-159
7	20.0	21	11.9-23.7	59-118

Table 55. Water intake in 7 healthy individuals on regular diet (3 days)

Subject	Water intake			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	40.0	17	32.4-45.5	81-105
2	22.8	18	18.4-26.8	85-124
3	20.8	13	18.1-23.4	100-129
4	21.4	34	15.7-29.8	73-139
5	14.2	22	10.7-16.7	76-118
6	22.4	18	18.7-26.8	97-119
7	20.0	33	15.0-27.4	75-137

Table 56. Water intake in 7 healthy individuals on
ovovegetarian diet (10 days)

Subject	Water intake			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	35.0	17	24.8-44.4	71-127
2	20.4	44	8.6-34.5	42-168
3	21.3	16	13.8-25.8	65-121
4	17.8	19	12.9-22.0	72-123
5	18.2	18	11.7-22.3	64-123
6	22.2	15	18.2-29.2	82-132
7	23.7	24	13.8-32.1	58-135

Table 57. Individual and mean($\pm$ SEM) water intake and urinary volume in 7 healthy men during the protein-free period (days 1 and 15)

Subjects	Water intake		Urinary volume	
	Day 1	Day 15	Day 1	Day 15
	<-----ml/kg/day ----->			
1	12.4	37.2	20.7	22.2
2	9.3	23.1	13.5	31.9
3	3.3	27.4	19.6	20.6
4	5.0	18.2	5.7	9.2
5	3.7	12.4	7.0	12.9
6	31.8	18.6	14.6	24.2
7	3.0	14.4	15.5	22.2
Mean $\pm$ SEM	9.8 $\pm$ 3.9	21.6 $\pm$ 3.2	13.8 $\pm$ 2.2	20.4 $\pm$ 2.8

Table 58. Mean($\pm$ SEM) water intake and urinary volume in 7 healthy men on various diets

Diet	Sample	Water intake		Urinary volume	
		ml/kg/day	ml/day	ml/kg/day	ml/day
BL	21	-	-	14.1 $\pm$ 1.8	993 $\pm$ 60
PF	14	15.7 $\pm$ 2.9	924 $\pm$ 169	17.1 $\pm$ 2.0	947 $\pm$ 135
VD	70	b1 25.9 $\pm$ 1.1	b1 1508 $\pm$ 63	a4 18.2 $\pm$ 0.9	a4 1036 $\pm$ 52
RD	21	b3 23.1 $\pm$ 1.9	b3 1352 $\pm$ 101	a3 19.5 $\pm$ 1.3	a3 1145 $\pm$ 67
OV	70	b2,c2 22.6 $\pm$ 0.9	b3,c1 1297 $\pm$ 47	a1 20.3 $\pm$ 0.8	a2 1161 $\pm$ 49
VD, RD, OV > BL: a1 a2 a3 a4 P<0.005, P<0.01, P<0.025, P<0.05 b1 b2 b3 VD, RD, OV > PF: P<0.001, P<0.005, p<0.05 c1 c2 OV < VD : P<0.01, p<0.025					

Table 59. Urinary volume in 7 healthy individuals during the last 3 days of baseline period

Subject	Urinary volume			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	19.6	24	15.2-24.4	78-125
2	16.5	41	8.8-22.0	53-133
3	11.8	43	6.2-16.0	53-136
4	12.3	22	9.4-14.4	76-117
5	13.4	14	12.0-15.6	90-116
6	11.8	36	7.4-15.9	63-135
7	13.5	44	8.9-20.1	66-149

Table 60. Urinary volume in 7 healthy individuals on vegetarian diet (10 days)

Subject	Urinary volume			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	29.7	32	15.6-45.9	53-155
2	22.9	30	11.9-31.4	52-137
3	17.7	24	11.9-24.1	67-136
4	12.4	15	9.0-14.9	72-120
5	14.8	15	13.6-19.5	91-131
6	15.9	18	12.2-22.4	77-141
7	13.8	30	10.3-24.4	75-177

Table 61. Urinary volume in 7 healthy individuals on regular diet (3 days)

Subject	Urinary volume			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	29.9	19	24.0-35.3	80-118
2	21.6	9	20.1-23.9	93-110
3	18.1	10	16.7-20.1	92-111
4	13.5	10	12.5-15.0	93-112
5	19.1	26	16.0-24.9	84-130
6	19.3	18	15.4-21.7	80-112
7	14.9	16	12.7-17.4	85-116

Table 62. Urinary volume in 7 healthy individuals on ovovegetarian diet (10 days)

Subject	Urinary volume			
	Mean	CV	Range	
	ml/kg/day	%	mean	% of mean
1	24.6	23	14.1-33.9	57-138
2	21.8	52	7.9-40.9	36-188
3	19.0	23	11.2-23.9	59-126
4	14.6	30	8.4-23.7	58-162
5	18.2	22	12.5-24.6	69-135
6	26.1	26	14.8-35.2	56-135
7	17.2	30	10.0-27.2	58-158

Table 63. Urinary creatinine excretion in 7 healthy individuals during the last 3 days of the baseline period

Subject	Urinary creatinine			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	16.5	16	14.3-19.5	87-118
2	10.9	24	9.3-14.0	85-128
3	14.3	43	7.2-18.0	50-126
4	18.5	22	15.6-21.3	85-116
5	13.1	40	9.0-18.9	69-145
6	12.8	25	12.5-20.1	98-157
7	18.3	49	11.1-28.3	61-155

Table 64. Urinary creatinine excretion in 7 healthy individuals during the vegetarian period (10 days)

Subject	Urinary creatinine			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	13.2	25	8.3-19.0	62-143
2	16.0	36	8.6-26.6	54-167
3	18.6	24	12.1-25.4	65-137
4	17.9	17	11.7-22.3	66-125
5	16.0	20	11.5-21.1	72-132
6	15.4	27	7.9-21.6	52-141
7	14.7	46	7.6-30.9	52-210

Table 65. Urinary creatinine excretion in 7 healthy individuals during the regular period (3 days)

Subject	Urinary creatinine			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	14.8	30	10.6-19.3	71-130
2	14.8	37	11.4-21.2	77-143
3	21.6	7	20.6-23.3	95-107
4	18.5	22	14.0-22.1	75-119
5	20.9	7	19.7-22.6	94-108
6	12.8	22	12.5-20.1	98-157
7	14.9	12	12.8-16.4	86-110

Table 66. Urinary creatinine excretion in 7 healthy individuals during the ovovegetarian period (10 days)

Subject	Urinary creatinine			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	14.8	23	9.6-19.6	65-132
2	12.9	41	5.2-22.0	40-170
3	18.4	20	10.9-23.1	59-126
4	19.1	15	13.4-23.2	70-122
5	21.7	33	13.9-36.4	64-168
6	12.1	34	4.8-18.2	40-150
7	16.4	66	5.2-33.7	32-205

Table 67. Individual and mean($\pm$ SEM) urinary creatinine in 7 healthy men during the protein-free period (days 1 and 15)

Subject	Urinary creatinine	
	Day 1	Day 15
	mg/kg/day	
1	7.6	8.0
2	14.8	17.3
3	18.4	23.3
4	12.3	16.5
5	2.3	13.4
6	8.4	17.8
7	10.5	33.6
Mean $\pm$ SEM	10.6 $\pm$ 2.0	18.6 $\pm$ 3.1

Table 68. Mean($\pm$ SEM) urinary creatinine in 7 healthy men on various diets

Diet	Sample	Urinary creatinine	
		mg/kg/day	mg/day
BL	21	15.52 $\pm$ 1.12	980 $\pm$ 79
PF	14	14.60 $\pm$ 3.90	910 $\pm$ 141
VD	70	16.00 $\pm$ 0.56	980 $\pm$ 41
RD	21	17.45 $\pm$ 0.87	1070 $\pm$ 62
OV	70	18.73 $\pm$ 2.39	1010 $\pm$ 50

Table 69. Individual and mean($\pm$ SEM) serum creatinine in 7 healthy men on various diets

Subject	Serum creatinine			
	BL	VD	RD	OV
	mg/dL			
1	0.97	1.15	1.06	0.97
2	1.45	1.36	1.15	1.15
3	1.21	1.45	1.21	1.45
4	1.21	1.21	1.15	1.06
5	1.15	1.27	1.15	1.15
6	1.45	1.27	1.45	1.36
7	1.30	1.27	1.21	1.15
Mean $\pm$ SEM	1.26 $\pm$ 0.06	1.28 $\pm$ 0.04	1.20 $\pm$ 0.05	1.18 $\pm$ 0.06 ^b

^b
OV < VD: P<0.05

Table 70. Individual and mean($\pm$ SEM) body surface area in 7 healthy men on various diets

Body surface area									
	BL	VD	RD	OV	BL	VD	RD	OV	
	$\frac{2}{m}$		$\frac{2}{m}$		$\frac{2}{m}$		$\frac{2}{m}$		
	$\langle \dots \rangle$		$\langle \dots \rangle$		$\langle \dots \rangle$		$\langle \dots \rangle$		% of 1.73 m
1	1.60	1.58	1.58	1.57	92	91	91	91	91
2	1.64	1.62	1.63	1.62	95	94	94	94	94
3	1.75	1.75	1.75	1.74	101	101	101	100	100
4	1.52	1.52	1.51	1.50	88	88	87	87	87
5	1.62	1.60	1.59	1.58	94	92	92	92	92
6	1.83	1.81	1.81	1.80	106	105	105	104	104
7	1.79	1.76	1.75	1.74	103	102	101	100	100
Mean $\pm$ SEM	1.68 $\pm$ 0.04	1.66 $\pm$ 0.04	1.66 $\pm$ 0.04	1.65 $\pm$ 0.04	97.0 $\pm$ 2.4	96.1 $\pm$ 2.4	95.8 $\pm$ 2.5	95.4 $\pm$ 2.3	a1, b2
VD, RD, OV < BL:	a1	a2	a3	c1	b1	b2	a3	a2	a1, b2
	P<0.005,		P<0.025,		OV < VD:		P<0.005,		P<0.05
OV < RD	c2								
	:		P<0.005						

Table 71. Individual and mean($\pm$ SEM) creatinine clearance in 7 healthy men on various diets

Subject	Creatinine clearance							
	BL	VD	RD	OV	BL	VD	RD	OV
	<----- ml/min ----->			<----- ml/min/1.73 m -----> ²				
1	68.4	47.6	56.8	60.1	74.0	52.1	62.2	66.2
2	35.2	45.6	54.0	49.6	37.2	51.9	57.6	53.0
3	52.2	54.6	87.7	61.7	57.6	63.9	86.7	61.0
4	55.6	57.1	60.2	69.8	63.3	65.0	69.0	80.6
5	46.8	51.1	75.2	73.6	50.0	55.3	81.8	80.6
6	60.3	59.2	53.8	46.0	57.0	56.6	51.4	44.2
7	66.9	52.2	58.3	63.2	64.7	51.3	57.6	62.8
Mean $\pm$ SEM	55.0 $\pm$ 4.4	52.5 $\pm$ 1.8	63.7 $\pm$ 4.8	60.6 $\pm$ 3.8	59.1 $\pm$ 5.5	56.6 $\pm$ 2.2	66.6 $\pm$ 5.0	64.0 $\pm$ 5.1

Discussion

Creatinine is a nitrogenous organic compound participating in cellular energy metabolism and found primarily in muscle. Body creatine is derived from diets and endogenous synthesis. Dietary creatine is mainly derived from muscle. The biosynthesis of creatine can be divided into 2 steps. The first step is the synthesis of guanidinoacetic acid from glycine and arginine (**Figure 1**).

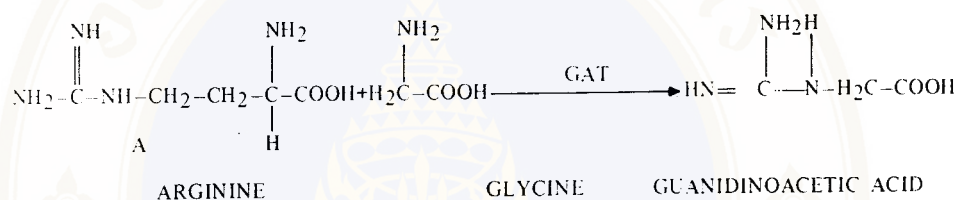


Fig 1. Formation of guanidinoacetic acid.

The reaction is catalyzed by the rate limiting enzyme glycine amidinotransferase (GAT) found in human kidney, liver, pancreas, brain, spleen, and mammary gland. However, the predominant biosynthetic site for guanidinoacetic acid in man is the kidney. The second step is the formation of creatine by transfer of a methyl group from S-adenosylmethionine to guanidinoacetic acid (**Figure 2**).

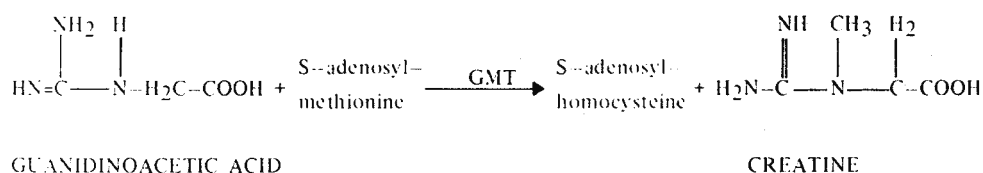


Fig 2. Formation of creatine.

This reaction is catalyzed by the enzyme guanidinoacetate methyl transferase (GMT) and require ATP. This second step is

irreversible, not rate limiting, and occurs primarily in the liver (49).

Biosynthesized creatine is released into the circulation and taken up by muscle and other tissues. The creatine uptake is an active process. It enters to the cell by binding to a specific membrane site which recognizes the formamidine moiety of the molecule. This active creatine uptake replaces about 2% of the total amount of creatine in muscle each day. About 98% of the total body creatine pool (120 g in a 70 kg adult man) is within muscle. This creatine pool is slowly saturable and has a relatively slow daily turnover rate of about 1.5 to 2%. Other tissues (brain, kidney, and liver), and fluids (blood and urine) contain measurable amounts of creatine. Because creatine in these tissues represent only a small percentage of the total body creatine pool, i.e., <2%, their over all contribution to creatine turnover is small (49).

Creatine uptake by muscle and other tissue is relatively complete, and blood levels rarely exceed 0.3 mg/dL. Urinary losses of creatine are usually negligible, unless blood levels become excessively high or renal tubular reabsorption is impaired.

Within muscle, creatine exists in two forms: creatine and phosphocreatine which is a high energy compound involving in excitation-contraction of muscle. The formation of phosphocreatine requires ATP and is catalyzed by the enzyme creatine kinase (Figure 3).

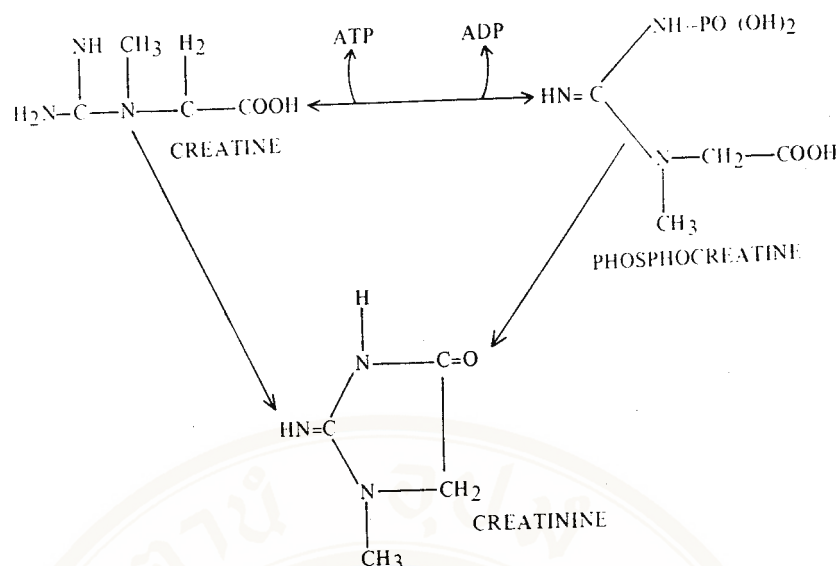


Fig 3. Formation of phosphocreatine and creatinine.

Creatine spontaneously dehydrates to creatinine at a rate of 1.1% per day while phosphocreatine conversion to creatinine is 2.64% per day (**Figure 3**). Once formed, creatine diffuses from the cell and ultimately appears in the urine after glomerular filtration, and to a small extent, tubular secretion (49).

Normally, urinary creatinine excretion varies daily, the causes of which are largely unknown. The constancy of 24-hr creatinine excretion reported in the early Folin studies, and later reinforced by Rose has been repeatedly challenged through the years (49). Careful studies in reliable subjects by Cryer and Sode (50) as well as Greenblatt et al (51) have shown a daily variation from 4 to 8% in creatinine excretion, which cannot be explained by minor variations in physical activity and diet. The factor affecting this urinary creatinine excretion in each individual is termed normal day to day variability with the magnitude of ± 4 to 8%. Factors which can increase daily urinary creatinine excretion other than the increase in muscle

mass are emotional stress with the magnitude of +5 to 10%, severe infection, high fever or trauma with the magnitude of +20 to 100%, second half of the menstrual cycle with the magnitude of +5 to 10%, and meat diets with the magnitude of +10 to 30%. On the other hand, decreased urinary creatinine excretion is observed in subjects on creatine-free diets and patients with impaired renal functions (49).

Three dietary factors, namely, protein, creatine, and creatinine, affect urinary creatinine excretion (49).

Dietary protein is the source of arginine, glycine, and methionine which are the precursors of biosynthesized creatine. Dietary protein intake influences GAT activity. Few studies have rigorously examined this relation independently from creatine and creatinine. In 1972, Bleiler and Schedl(52) studied six men on natural diets containing 120 g protein and the usual amount of dietary creatine (basal diet). These subjects were divided into 3 groups and switched to a creatine-free diet; the two subjects in each group received 140, 70, and 20 g of protein, respectively, for 11 wk. They were then switched to a creatine-free diet containing 120 g protein for 3 wk. The mean urinary creatinine excretions in 2 subjects on creatine-free 20 g protein diet were 20.0, 17.6-12.0, and 12.8-13.6 mg/kg/day during receiving, the basal diet, creatine-free 20 g protein diet, and creatine-free 120 g protein diet, respectively. The corresponding mean urinary creatinine excretions in 2 subjects on creatine-free 70 g protein diet were 25.3,

22.3-17.2, and 18.3-19.0 mg/kg/day whereas those in 2 subjects on creatine-free 140 g protein diet were 24.7, 22.1-18.2, and 18.8-19.6 mg/kg/day. Over wk 8-11 of creatine-free 20, 70, or 140 g protein diets, urinary creatinine excretion fell in each of the respective groups by 40, 32, and 26% whereas over 3 wk of creatine-free 120 g protein diet, the urinary creatinine excretions in the 3 groups increased by 13, 10, and 7.7%, respectively.

The effect of level of protein intake on urinary creatinine excretion has also been reported by Bosch et al (53). Three men and 2 women with the mean age of 31 yr and body weight of 58.6 kg were fed three levels of protein diets, namely, 40, 70, and 90 g protein diets consecutively. Each diet was fed for 7 days. Details of the diet were not provided by the authors. Urinary creatinine was measured from a 24-hr urine collected at the end of each period. The mean($\pm$ SEM) urinary creatinine excretions were $1,345 \pm 137$, $1,475 \pm 128$, and $1,590 \pm 151$ mg/day during receiving 40, 70, and 90 g protein diets, respectively. The corresponding urinary creatinine excretions in mg/kg/day were 23, 25, and 27. Urinary creatinine excretions during receiving 70 and 90 g protein diets were significantly higher than that during receiving 40 g protein diet.

The influence of arginine and glycine intake has been reported by Crim et al (54) who fed normal volunteers

a creatine-free purified formula containing 75 g egg albumen for 71 days, adding during the last 10 days isonitrogenous amounts of 4 g nitrogen daily from either and equimolar mixture of arginine and glycine or alanine. There was a small but significant increase in urinary creatinine excretion in 2 of the 4 subjects fed arginine-glycine, i.e., 1,640 vs 1,680 and 1,850 vs 1,920 mg/day. No significant changes were observed in the other two subjects supplemented with arginine-glycine, i.e., 1,920 vs 1,940, and 1,910 vs 1,910 mg/day, or in another two subjects supplemented with alanine, i.e., 2,050 vs 2,040 and 1,830 vs 1,840 mg/day.

Several investigators have examined the influence of dietary creatine on urinary creatinine excretions. Bleiler and Schedl (52) showed that switching from an ad libitum diet to a meat-free protein source (casein) there was a reduction in urinary creatinine excretion over 6 wk from 1.9 to 1.5 g/day (-21%). They also demonstrated a rapid increase in urinary creatinine excretion from a creatine-free 120 g protein diet to an ad libitum natural diet, 14 vs 20 mg/kg/day. Crim et al (55) shifted from a 9-day low creatine diet (0.23 g/day), to a 10-day high creatine diet (10 g/day), and found a prompt 10 to 30% increase in urinary creatinine excretion. In a subsequent creatine-free period, Crim et al (55) showed that nitrogen and potassium balance remained positive despite declining

creatinine excretion, indicating a degree of independence between creatinine excretion, and muscle or lean body mass. The independence between creatinine excretion and muscle or fat free body mass is also observed when switching from a general hospital diet which includes meat, to a creatine-free hyperalimentation formula and vice versa.

Bosch et al (53) also showed that the mean($\pm$ SEM) urinary creatinine excretion in 5 subjects on their usual diets was $1,519 \pm 135$ mg/day or 26 mg/kg/day whereas this parameter in 1 male and 7 female vegetarians defined by avoiding consumption of red meat for at least 12 months was 831 ± 101 mg/day or 13 mg/kg/day which was significantly lower than that in the former group.

The third dietary factor influencing urinary creatinine excretion is creatinine. Dietary creatinine undergoes intestinal absorption and prompt renal excretion (49).

Our study indicates that the daily urinary creatinine excretion can vary not only among different subjects but also in the same subject from one day to another, regardless of diets (Tables 63-66). Our results agree with other reports which indicate that the daily urinary creatinine excretion cannot be used as a reliable index of the completeness of urine collection (56,57). It should be pointed out that urinary volume should not be the

factor causing variation of urinary creatinine excretion in our study because there was no correlation between urinary volume and urinary creatinine excretion (Tables 58, 68).

Most of human water intake, 1,000-1,500 ml/day, comes directly through drinking water and other beverages. About 700 to 1,000 ml of daily intake comes from the food consumed. For most adults the total daily intake from all sources averages 2,100-2,500 ml (58).

Urine volume represents the difference between total water intake plus water of oxidation and the sum of insensible losses plus the water in stool of 100-200 ml/day. Normal adults excrete urine 1,000-2,500 ml/day whereas minimal urinary volume in young adults with normal renal function is about 600 ml/day (59).

Based on the aforesaid knowledge of water intake and excretion, the data in Table 58 indicate that our subjects during the PF, VD, RD, and OV periods were in positive water balances and had normal renal function. Besides, we had observed significant positive correlation between water intake in ml/day and urinary volume in ml/day ($y=463.978+0.495x$, $r=0.6510$, $df=19$, $t=3.7381$, $P<0.005$). It should be pointed out that during each dietary period the amount of protein, fat, and carbohydrate consumed daily by each subject was held constant. Thus the amount of daily water oxidation in each subject

during each dietary period should not vary considerably. The oxidation of each gram of protein, fat, and carbohydrate provides 0.41, 1.07, and 0.6 ml of water (59). Based on these figures the means of estimated amount of water derived from the oxidation of food were 280, 266, 205, and 266 ml during the PF, VD, RD, and OV periods. These indicate that the variation of water of oxidation among these 4 dietary periods were less than that of water intake **Table 58**.

In our study, VD, OV, and PF were creatine-free diets whereas BL and RD contained creatine. Urinary creatinine excretion during the VD and OV periods were not significantly different (**Table 68**) because during these 2 dietary periods the subjects had isocaloric and isonitrogenous intake. The lack of significant difference in urinary creatinine excretion between the BL and VD or OV periods could be explained by the lower protein intake during the BL period than the VD and OV periods and the presence of creatine in the BL diet. During the RD period, the subjects had creatine intake and higher protein intake than the VD and OV periods which explained higher urinary creatinine excretion during the RD period than the VD and OV periods. However, the difference did not reach statistical significance which was most likely due to the short duration of the RD period (3 days). This was supported by the lowest urinary creatinine excretion

during the PF period (days 1 and 15) but it was not significantly different from urinary creatinine excretions in the other periods.

It seems that our results were contradictory to those of Bosch et al (53) and Bleiler and Schedl (52). However, this is not the case after we have examined their data carefully. The effect of the levels of protein intake on urinary creatinine excretion reported by Bosch et al (53) was seen only between 40 g and 70 g or 90 g meat protein diet whereas no difference in urinary creatinine excretion was found between 70 g and 90 g meat protein diet. Since their protein diets were not creatine-free the change in urinary creatinine excretion also depended on the amount of creatine intake which, in turn, was related to the levels of meat protein intake. The effect of creatine intake instead of protein intake on urinary creatinine excretion was evident by the study of Bleiler and Schedl (52) who showed that after switching from 120 g protein diet to creatine-free 140 g, 70 g, or 20 g protein diets the decline in urinary creatinine excretion was observed to the same extent during the first week of these creatine-free diets, i.e., 10, 11, and 12%, respectively. The decreases in urinary creatinine excretion during these creatine-free diets reached the maximum at 8 wk, i.e., 30, 32, and 40%. This indicates that creatine intake has more influence than the level of protein intake on urinary

creatinine excretion. Thus it can be concluded that the level of protein intake per se appears to have a small effect on urinary creatinine excretion.

Since creatinine is a by-product of creatine metabolism residing in skeletal muscle, 24-hr urinary creatinine excretion is considered most reliable of the measurements used to assess the degree of depletion of muscle mass. In the presence of renal function, the urinary creatinine excretion does reflect the amount of skeletal muscle, i.e., 17 to 20 kg/g urinary creatinine (49). Our results support the relationship between urinary creatinine excretion and muscle mass evidenced by significant positive correlation between urinary creatinine excretion in g/day and fat-free mass in kg ($y=30.680+0.154x$, $r=0.5927$ $df=26$, $t=3.7523$, $P<0.001$) as well as between urinary creatinine excretion in g/day and BMI in kg/m^2 ($y=18.467+4.681x$, $r=0.5420$, $df=26$, $t=5.5406$, $P<0.001$).

Bristrian et al (60) reported the creatinine coefficient derived from g urinary creatinine/kg body weight is 18-21 mg/kg for American women and 21-25 mg/kg for American men. The creatinine coefficient in our healthy men on the BL diet was 15 mg/kg. The lower creatinine coefficient in our subjects than that in American subjects should reflect lower muscle mass and lower meat protein intake in our subjects.

The finding that creatinine is concentrated in the urine more than most other endogenous substances eliminated by the kidneys led to the use of creatinine as a test of renal function or more specifically the measurement of glomerular filtration rate (GFR). Two main procedures are used for the measurement of creatinine clearance, i.e., short creatinine clearance by collecting the urine for a period of 1 or 2 hr and long creatinine clearance by collecting the urine over a period of 24 hr. In both procedures blood samples are usually drawn at the end of the study for plasma creatinine determination. The long creatinine clearance has been considered to be a more physiological index of GFR because factors affecting GFR, e.g., physical activity, drug effect, emotional stress, dietary intake, and changes in intraabdominal and venous pressure are included in the clearance determination (61).

Normal values for serum creatinine are 0.9-1.5 mg/dL in men and 0.7-1.3 mg/dL in women (20). Serial measurements taken throughout the day in adults revealed that creatinine clearance decreased between 12.00 p.m. and 4.00 a.m., the deepest sleep hours. In samples taken at 4-hr interval, a 5-17% variation in serum creatinine was detected which were not related to creatinine clearance. Serum creatinine was higher by 11% in the afternoon and urinary creatinine excretion was elevated in the evening hours. Fasting eliminated or minimized these variations

in plasma serum and urinary creatinine concentration. Thus it is recommended that blood sample for serum creatinine determination should be drawn in the morning under fasting condition (61).

In our study, blood samples for serum creatinine determination were drawn in the morning under fasting condition at the end of each dietary period. Urinary creatinine concentration in each subject was determined daily during each dietary period and the mean urinary creatinine concentration in each subject during each dietary period was used for the calculation of creatinine clearance. The same principle was applied for urinary volume. Thus it was a modified long creatinine clearance. Our subjects had normal serum creatinine concentration throughout the study (**Table 69**). Since VD and OV diets were creatine-free the body creatine of the subjects depended on endogenous synthesis of creatine. The higher serum creatinine level during the VD period than during the OV period (**Table 69**) was possibly due to higher intake of arginine and glycine during the VD period than during the OV period (**Tables 15 and 16**) and/or lower urinary creatinine excretion during the VD period.

The reference values of creatinine clearance in the literature vary considerably. The values cited by Tietz (20) are $97-137 \text{ mL/min/1.73 m}^2$ for men and $88-128 \text{ mL/min/1.73 m}^2$ for women with the aged less than 40 yr;

the creatinine clearance decreases $6.5 \text{ mL/min/1.73 m}^2$ per decade. The values cited by Young (47) are 75-125 mL/min without age and sex specification and the values are not adjusted to 1.73 m^2 . The values of creatinine clearance reported by Duarte et al (61) were 81-119 mL/min/ 1.73 m^2 in 4 men aged 26-46 yr and 87-139 mL/min/ 1.73 m^2 in 3 women aged 21-25 yr; creatinine clearances in these 7 subjects were derived from daily collection of 24-hr urine specimens for 31 days. Bosch et al (53) reported creatinine clearances in 3 men on different levels of protein intake: the first subject aged 29 yr had the creatinine clearances of 95, 92, 95, and 136 mL/min/ 1.73 m^2 during receiving ad libitum diet, 40, 70, and 90 g protein diets, respectively, the corresponding values in the second subject aged 35 yr were 125, 117, 127, and 140 mL/min/ 1.73 m^2 , and the corresponding values in the third subject aged 38 yr were 114, 98, 103, and 114 mL/min/ 1.73 m^2 . They also reported the creatinine clearances in 1 man and 7 women aged 26-36 yr on vegetarian diet for at least 1 yr ranged from 29-94 with a mean of $68 \text{ mL/min/1.73 m}^2$. Thus it is evident from these reports that creatinine clearance in healthy adults vary considerably. Our results are consistent with these reports (Table 71). The factor causing the variation in creatinine clearance in healthy subjects is urinary creatinine concentration which can be affected by muscle mass and meat protein intake.

CHAPTER V

INFLUENCE OF SOYBEAN COMPARED WITH SOYBEAN-EGG DIETS
ON URIC ACID METABOLISM

Results

Urinary uric acid

Tables 72-75 show urinary uric acid excretions in 7 subjects expressed as mg/kg/day, its CV, range in mg/kg/day and percentage of mean during the BL, VD, RD, and OV periods. The CV of uric acid excretion during the VD and OV periods were less than those during the BL and RD periods. Table 76 shows urinary uric acid excretion during the PF period in these subjects. Using the same principle in calculating mean urinary protein excretion (Table 37), Table 77 shows the mean($\pm$ SEM) urinary uric acid excretion of 7 subjects during the various diets. Urinary uric acid excretion in mg/kg/day during the PF period was significantly lower than those during the BL, VD, and RD periods, whereas urinary uric acid excretion during the RD period was significantly higher than that during the OV period. Urinary uric acid excretion in mg/day during the RD period showed the highest value and was significantly higher than those during the other periods, whereas urinary uric acid excretion during the VD period was significantly higher than that during the OV period.

Serum uric acid and uric acid clearance

Individual and mean($\pm$ SEM) serum uric acid in 7 subjects on various diets are shown in Table 78. The mean($\pm$ SEM) serum uric acid levels during the BL and RD periods were significantly higher than those during the VD and OV periods.

Table 79 shows individual and mean($\pm$ SEM) uric acid clearance in these subjects on various diets. During the BL period uric acid clearance was significantly higher than those during the other periods.

Table 80 shows that the mean daily purine and ribonucleic acid intake during the VD period were significantly higher than those during the OV period.

Discussion

In humans, uric acid is the major product of the catabolism of the purine nucleosides, adenosine and guanosine. Purines from catabolism of dietary nucleic acid are converted to uric acid directly. However, the bulk of purines ultimately excreted as uric acid in the urine arises from degradation of endogenous nucleic acids. The metabolism of purines is illustrated in Figure 4 (62). Reutilization of the major purine bases, adenine, hypoxanthine, and guanine is achieved through salvage pathways in which phosphoribosylation of the free bases causes resynthesis of the respective nucleotide monophosphates.

Table 72. Urinary uric acid excretion in 7 healthy individuals during the last 3 days of the baseline period

Subject	Urinary uric acid			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	11.4	18	19.0-13.1	79-115
2	5.9	29	4.8-7.8	82-133
3	4.7	51	2.8-7.4	60-158
4	8.8	34	6.9-12.3	78-140
5	6.6	25	5.0-8.3	76-126
6	5.5	26	4.2-7.0	77-128
7	6.0	23	4.5-7.0	74-118

Table 73. Urinary uric acid excretion in 7 healthy individuals during the vegetarian period (10 days)

Subject	Urinary uric acid			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	11.0	14	9.0-12.8	82-116
2	7.3	21	5.2-10.2	72-140
3	5.1	12	3.9-6.0	77-118
4	8.9	19	6.6-11.9	74-134
5	7.5	19	4.8-9.8	64-130
6	6.3	19	4.0-8.2	64-130
7	8.8	17	6.7-10.7	77-123

Table 74. Urinary uric acid excretion in 7 healthy individuals during the regular period (3 days)

Subject	Urinary uric acid			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	16.9	30	13.7-22.8	81-134
2	9.4	29	6.8-12.2	73-130
3	6.6	11	5.8-7.1	87-106
4	8.8	34	6.9-12.3	78-140
5	20.9	7	19.7-22.6	94-108
6	8.0	26	6.8-10.4	85-130
7	10.9	30	7.1-13.1	65-120

Table 75. Urinary uric acid excretion in 7 healthy individuals during the ovovegetarian period (10 days)

Subject	Urinary uric acid			
	Mean	CV	Range	
	mg/kg/day	%	mg/kg/day	% of mean
1	10.7	28	7.0-16.3	66-152
2	6.0	20	3.6-7.6	60-126
3	4.8	14	3.6-5.4	74-113
4	8.2	20	6.6-12.3	81-150
5	6.7	15	5.0-8.2	75-123
6	5.5	15	4.1-6.8	75-123
7	7.9	17	5.5-9.6	70-122

Table 76. Individual and mean($\pm$ SEM) urinary uric acid in 7 healthy men during the protein-free period (days 1 and 15)

Subject	Urinary uric acid	
	Day 1	Day 15
	mg/kg/day	
1	7.6	8.7
2	4.6	5.5
3	5.8	4.8
4	4.3	4.8
5	8.1	4.2
6	6.9	3.5
7	7.0	5.9
Mean $\pm$ SEM	6.3 $\pm$ 0.6	5.3 $\pm$ 0.6

Table 77. Mean($\pm$ SEM) urinary uric acid in 7 healthy men on various diets

Diet	Samples	Urinary uric acid	
		mg/kg/day	mg/day
BL	21	8.24 $\pm$ 0.86	440 $\pm$ 32
PF	14	5.85 $\pm$ 0.43 ^{a2}	350 $\pm$ 26 ^{a3}
VD	70	7.84 $\pm$ 0.27 ^{b3}	480 $\pm$ 14 ^{b2}
RD	21	9.52 $\pm$ 0.98 ^{b3}	640 $\pm$ 45 ^{a1,b1,c}
OV	70	7.10 $\pm$ 0.28 ^{d2}	410 $\pm$ 16 ^{c,d1}
RD > BL: P<0.005,		PF < BL: P<0.025,	P<0.05
VD, RD > PF: P<0.0001,		P<0.001,	P<0.005
RD > VD, OV < VD: P<0.005			
OV < RD, P<0.0005,		P<0.025	

Table 78. Individual and mean($\pm$ SEM) serum uric acid in 7 healthy men on various diets

Subject	Serum uric acid			
	BL	VD	RD	OV
	mg/dL			
1	7.26	5.89	6.10	5.36
2	5.20	4.32	4.97	3.84
3	5.50	5.59	6.10	5.08
4	4.41	3.97	4.78	3.69
5	4.11	3.86	5.01	4.04
6	6.45	5.29	5.36	5.22
7	2.73	2.40	3.37	2.35
Mean $\pm$ SEM	5.09 $\pm$ 0.57	4.47 $\pm$ 0.46 ^a	5.10 $\pm$ 0.35 ^b	4.23 $\pm$ 0.41 ^{a,c}
^a VD,OV < BL : P<0.025, ^b RD > VD : P<0.01, ^c OV < RD : P<0.001				

Table 79. Individual and mean($\pm$ SEM) uric acid clearance in 7 healthy men on various diets

Subject	Uric acid clearance							
	BL	VD	RD	OV	BL	VD	RD	OV
	<----- ml/min ----->				<----- ml/min/1.73 m ² ----->			
	<----- ml/min ----->				<----- ml/min/1.73 m ² ----->			
1	6.26	7.83	10.52	7.67	6.77	8.58	17.17	13.67
2	5.21	7.29	7.87	7.79	5.50	7.78	8.35	8.32
3	4.53	4.76	5.36	4.79	4.48	4.71	5.30	5.77
4	7.75	8.57	9.81	8.57	8.83	9.76	11.24	9.88
5	6.95	7.89	7.63	6.79	7.43	8.53	8.31	7.43
6	4.53	5.87	7.21	5.45	4.28	5.61	6.89	5.24
7	11.28	17.74	14.75	15.98	10.90	17.44	14.58	15.89
Mean _± SEM	6.64 _± 0.90	8.56 _± 1.61	9.00 _± 1.15	8.15 _± 1.40	6.88 _± 0.91	8.9 _± 1.57	17.1 _± 6.15	9.31 _± 1.58
	a2	a1	a2	a1	a2	a2	a2	a2

Table 80. Individual and mean($\pm$ SEM) daily purine intake from soybean in 7 healthy men on vegetarian and ovovegetarian diets

Subject	Purine	
	VD	OV
	<---- mg/day ---->	
1	479	385
2	505	404
3	470	378
4	510	408
5	497	397
6	630	504
7	533	426
Mean $\pm$ SEM	518 $\pm$ 20	414 $\pm$ 16 ^a

^a
OV < VD: P<0.0001

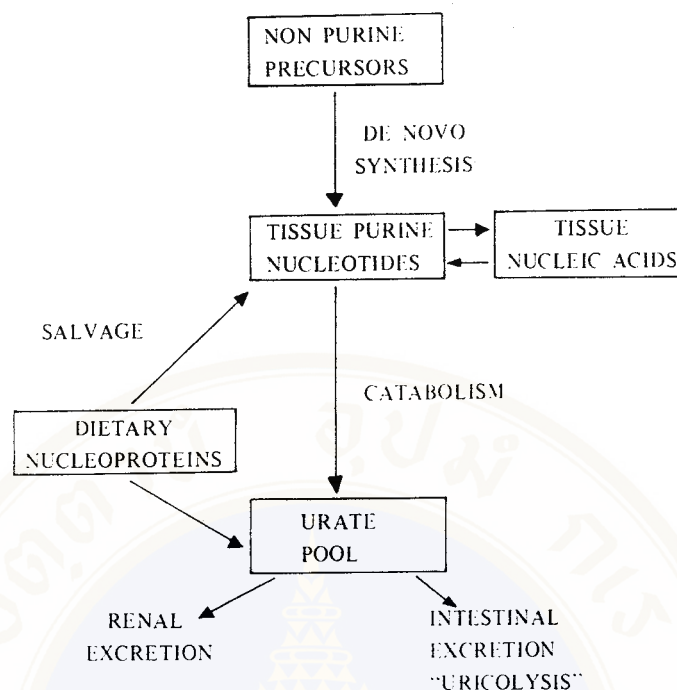


Fig 4. Purine metabolism⁽⁶²⁾.

Approximately 75% of uric acid excreted is lost in the urine; most of the remainder is secreted into the gastrointestinal tract, where it is degraded to allantoin and other compounds by bacterial enzymes. Renal handling of uric acid involves glomerular filtration, reabsorption in the proximal convoluted tubule, subsequent secretion of uric acid into the distal portion of the tubule, and further reabsorption in the distal tubule. The net urinary excretion of uric acid is 6-12% of the amount filtered (20).

Hyperuricemia is most commonly defined by serum or plasma uric acid concentrations ≥ 6.2 mg/dL in men or ≥ 5.1 mg/dL in women (63). The major causes of hyperuricemia are essential hyperuricemia: overproduction (associated with hyperaciduria), underexcretion (associated with

normal or decreased renal excretion of uric acid); renal retention, e.g., renal failure, drug therapy, (diuretics, salicylates), poisons (lead, alcohol); increased turnover of nucleic acids; and specific enzyme defects (hypoxanthine-guanine phosphoribosyl transferase, abnormal phosphoribosyl pyrophosphate synthetase). Asymptomatic hyperuricemia is frequently detected through biochemical screening; long-term follow-up of asymptomatic hyperuricemic subjects is undertaken because many are at risk for renal disease that may develop as a result of hyperuricemia and hyperuricosuria. Besides, several studies have shown that hyperuricemia is a minor risk factor of coronary heart disease (63).

Serum and urinary uric acid are influenced by the nucleic acid and protein content in the diet. Purines in the diets on a weight basis will cause a greater increase in uric acid excretion than will dietary proteins. A high purine diet, normally obtained from substantial meat intake, increases the urinary uric acid by approximately 0.5-0.75 mg/mg of ingested purine (64-67).

When the level of dietary protein is increased the serum uric acid level is lower and the urinary uric acid is higher without an appreciable of urinary creatinine excretion (64,68). A decrease in serum uric acid level of 0.086 mg/dL and an increase urinary uric acid of 7 mg/day was found for every 10 g of protein ingested, based on

2,400 kcal diet (68). This has been related to an increased renal clearance of uric acid with protein consumption (69,70), although some studies have shown an increased endogenous production of uric acid at higher levels of dietary protein (69). The ingestion of amino acids such as alanine, aspartic acid, glutamic acid, or glycine will increase uric acid excretion, possibly by decreasing uric acid reabsorption in the renal tubule (71,72). Uric acid excretion increased from 218-436 mg/day in adults when the dietary protein increased from 0 g/day to 63-75 g/day (64). Increased protein ingestion is small increments of 22 to 37 to 75 g/day of egg albumin by healthy male volunteers did not change the average urinary excretion and plasma urate (64).

Unlike some studies employing formula diets with or without nucleic acid, natural protein sources were used in our study. Thus our diets contained both protein and nucleic acid. The influence of dietary protein and nucleic acid intake on urinary uric acid level is also evident in our study by the findings that the highest urinary uric acid excretion was detected during the highest protein intake of the RD period whereas the lowest urinary uric acid excretion was found during the PF period (Table 77) as well as the significant positive correlation between protein intake and urinary uric acid excretion ($y=0.325+0.002x$, $r=0.5536$, $df=26$, $t=3.3981$, $P<0.001$).

However, though VD and OV diets were isocaloric and isonitrogenous urinary uric acid excretion during the VD period was significantly higher than that during the OV period (Table 77). This was due to the significantly higher purine intake during the VD period than during the OV period (Table 80). The only difference between VD and OV diets was the partial replacement of soybean by egg, a purine-free food, in the OV diet. Our finding that purine intake also influence urinary uric acid excretion is consistent with the reports of Edozien et al (65) and Coe et al (73).

The positive effect of the levels protein intake on uric acid clearance is also evident in our study. The uric acid clearances during the RD, VD, and OV periods were significantly higher than that during the BL period (Table 79). This explains why serum uric acid levels during the RD and BL periods were not significantly different (Table 78). However, the higher serum uric acid level during the RD period than during the VD and OV periods was probably due to the higher purine intake derived from the chicken protein during the RD period than during the VD and OV periods. This was supported by the lack of significant difference in uric acid clearance between the RD and VD or OV periods (Table 79).

It should also be noted that 2 subjects during the BL period were hyperuricemia. Their serum uric acid

levels became normal during the VD, RD, and OV periods. This was most likely due to lower purine intake during the study than their habitual diets. Coe et al (73) had reported that patients with calcium oxalate stone formers had higher purine intake than normal subjects.



CHAPTER VI

SUMMARY

This study was carried out to investigate the influence of soybean compared with soybean-egg diets on protein-calorie status and protein metabolism.

Seven healthy males, aged 30-35 yr, participated in a 25-day metabolic study. They received the following diets: protein-free diet (PF) for days 1 and 15, vegetarian diet (VD) for days 2-11, regular diet (RD) for days 12-14, and ovovegetarian diet (OV) for days 16-25. Energy intake for each subject receiving PF, VD, or OV was held constant according to his energy intake during the baseline period plus 1 SD whereas RD was served ad libitum. Energy distributions of VD and OV were 15, 30, and 55% derived from protein, fat, and carbohydrate, respectively. The protein sources of VD were soybean (90%) and rice (10%) whereas those of OV were soybean (72%), egg (18%), and rice (10%).

Blood was collected after a 12-hr fast at the following intervals together with the anthropometric measurements: day 1, before receiving PF diet; day 12, at the end of the VD period; day 15, at the end of the RD period; and day 26, at the end of the OV period for hematological and biochemical determination.

Twenty-four hr urine samples were collected during the last 3 days of the BL period and the 25 days of metabolic period. Urinary total nitrogen, creatinine and uric acid were analysed from each sample of each subject. Pooled stool collections at the last 5 days of VD and OV periods were analyzed for total nitrogen.

Nitrogen balance, urinary creatinine clearance, and uric acid clearance were calculated in each period of each subject.

The results of the study are summarized into 3 parts, i.e., protein-calorie status, creatinine metabolism, and uric acid metabolism.

Protein-calorie status

During the VD and OV periods, the subjects were satiated and satisfactory with food menus. This was evident by higher energy intake during these periods than the RD period. However, their mean body weight and BMI decreased significantly during the study because their energy intake during the PF, VD, OV, and RD periods were lower than the estimated energy requirement. The anthropometric measurement revealed that the decrease in their body weight was mainly due to the loss of adipose tissue.

The mean protein intake during the VD and OV periods were isonitrogenous and higher than the calculated safe levels of protein intake. The indispensable amino

acid (IDAA) intake during these 2 periods were also higher than the newly revised estimation of IDAA requirement for adults. The IDAA intake constituted 38.6 and 39.8% of total amino acids. Thus the protein sources of VD and OV were quantitatively and qualitatively adequate. This was supported by non-significant difference in nitrogen balances between these two periods. This indicates that substituting soybean protein by egg protein for 18% while total nitrogen and energy intake being kept constant no further increase in positive nitrogen balance was observed. This is consistent with (a) the similar figures of apparent digestibility, biological value, and net protein utilization of VD and OV, and (b) the anthropometric data which revealed the preservation of the somatic protein status evidenced by no significant loss in body muscle mass, upper arm muscle circumference, and bone-free arm muscle area during the study.

The high fecal weight and nitrogen excretion during the VD and OV periods were most likely due to the effect of dietary fiber present in these diets. Our study has also shown that the measurement of urinary total nitrogen excretion is useful for assessing nitrogen intake.

Regarding to visceral protein status, all of the subjects had serum total protein and albumin levels in the acceptable levels throughout the study. However, their mean serum transferrin levels during the study tended to

be lower than that during the BL period which was most likely due to inadequate energy intake with sufficient protein intake. The decrease in serum RBP levels during the study was possibly due to low vitamin A intake derived from carotene only during the VD period and mainly during the OV period.

Our subjects exhibited normal serum urea nitrogen levels and urinary protein excretions throughout the study. This implies their normal renal function.

Regarding to hematological status, the subjects were not anemic and had normal lymphocyte count which implied adequate cell-mediated immune response.

Creatinine metabolism

Our study indicates that the daily urinary creatinine excretion can vary not only among different subjects but also in the same subject from one day to another regardless of diets. There was no correlation between urinary volume and urinary creatinine excretion. Thus urinary volume should not be the factor causing variation of urinary creatinine excretion.

During the VD and OV periods, the subjects had creatine-free, isocaloric and isonitrogenous diets. Thus their urinary creatinine excretions during these 2 periods were not significantly different. Though the protein intake during the BL period was lower than that during the VD and OV periods the protein intake did not affect the

urinary creatinine excretion. This was most likely due to the presence of creatine in the BL diet. However, the influence of protein intake on urinary creatinine excretion was observed during the RD period but the increase in urinary creatinine excretion did not reach statistical significance because of the short duration of the RD period (3 days). This indicated that creatine intake has more influence than the level of protein intake on urinary creatinine excretion. Thus it can be concluded that the level of protein intake per se appears to have a small effect on urinary creatinine excretion.

Our results support the relationship between urinary creatinine excretion and muscle mass evidenced by significant positive correlation between urinary creatinine excretion and fat-free mass as well as between urinary creatinine excretion and BMI. The creatinine coefficient in our healthy men on the BL diet was 15 mg/kg. The lower creatinine coefficient in our subjects than that in American subjects should reflect lower muscle mass and lower meat protein intake in our subjects.

Since VD and OV diets were creatine-free the body creatine of the subjects depended on endogenous synthesis of creatine. The higher serum creatinine level during the VD period than during the OV period was possibly due to higher intake of arginine and glycine during the VD period than during the OV period and/or lower urinary creatinine

excretion during the VD period. Creatinine clearance in our subjects varied considerably throughout the study. It was caused by the variation in urinary creatinine excretion which was affected by muscle mass and meat protein intake.

Uric acid metabolism

Our diets contained both protein and nucleic acid. The influence of dietary protein and nucleic acid intake on urinary uric acid level is also evident in our study by the findings that the highest urinary uric acid excretion was detected during the highest protein intake of the RD period whereas the lowest urinary uric acid excretion was found during the PF period as well as the significant positive correlation between the protein intake and urinary uric acid excretion. The only difference between VD and OV diets was the partial replacement of soybean by egg, a purine-free food, in the OV diet. Thus the purine intake during the VD period was significantly higher than that during the OV period which resulted in significantly higher urinary uric acid excretion during the VD period than the OV period.

The positive effect of the levels protein intake on uric acid clearance is also evident in our study. The uric acid clearances during the RD, VD, and OV periods were significantly higher than that during the BL period.

Thus it can be concluded that soybean protein intake at 13.5% of total dietary energy did not cause hyperuricemia and uricosuria.

Conclusion

Protein is an essential nutrient throughout life. Though vegetable proteins are limiting in one or more of indispensable amino acids vegetables are of considerable importance in supplying the world's protein needs. Vegetable proteins can be improved in its quality by using a variety of legumes and seeds. Because of the abundance and low cost of vegetable proteins, they played the important role in protein nutrition for various populations, especially in the developing countries. Our studies presented here have shown the benefits of soybean complementation to rice-based diet, when it is used as the major dietary protein source. Unlike other studies using soy protein isolate or soy protein concentrate, we employed natural soybean. Thus our menus can be recommended to free-living population to replace partially their meat diets and to persons who want to consume vegetarian diets for a short-term of 7-10 days. Our diets seem to be also appropriate for obese subjects in order to reduce their body weight. However, the long-term use of our diets in subjects with normal weight it may be necessary to increase their energy intake in order to avoid weight loss.

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