AN ABSTRACT OF THE DISSERTATION OF

Abdullah Al-Assaf for the degree of Doctor of Philosophy in Nutrition and Food Management presented on August 11, 2003. Title: Evaluation of Vitamin B-6 Status of Saudi Adult Males in the Riyadh Region – Saudi Arabia.

Abstract approved: _____

James E. Leklem

The aim of this study was to investigate the vitamin B-6 status of Saudi adult males and compare the status between rural and urban subjects. Fifty-one adult male subjects were recruited from urban (n=31) and rural (n=20) populations of Riyadh. These subjects were reclassified to cigarette smokers (n=19), water pipe smokers (n=5) and non-smokers (n=27). The study also investigated the intake of macronutrients and selected micronutrients. In addition, the study investigated other health indicators including Body Mass Index (BMI), hematocrit, hemoglobin, plasma alkaline phosphatase activity and albumin concentration, urinary creatinine and urea nitrogen excretion.

The mean of vitamin B-6 intake, B-6 to protein ratio, plasma pyridoxal phosphate (PLP) concentration and urinary 4-PA excretion in urban group were 2.18± 0.62 mg/day, 0.022± 0.008 mg/g, 39.3± 18.0 nmol/L and 4.6± 2.3 μmol/day, respectively.

In rural group, these measures were 2.15± 0.65 mg/day, 0.021± 0.004 mg/g, 40.5± 14.6 nmol/L and 4.4± 2.3 μmol/day, respectively. These measures indicated adequate status with no significant difference between the two groups. The mean intake of calcium, folate, vitamin D, zinc and dietary fiber was lower than recommendation of the Dietary Reference Intakes (DRI) in both groups. Health indicators were within normal range except for BMI, which indicated a prevalence of overweight and obesity in both urban (27.1± 5.5 Kg/m^2) and rural (28.2± 6.0 Kg/m^2) subjects.

Comparison of the three smoking groups showed that the water pipe smokers compared to cigarette smokers and non-smokers groups had significantly higher mean intake of vitamin B-6 (2.51± 0.73 mg/day), which resulted in higher concentrations of plasma PLP, pyridoxal (PL), red blood cells PLP and urinary 4-PA (54.9± 23.1 nmol/L, 21.5± 10.0 nmol/L, 33.7± 8.5 nmol/L and 6.9± 4.7 μmol/day, respectively). Cigarette smokers had significantly lower concentration of plasma PLP (30.9± 12.5 nmol/L) compared to non-smokers (40.0± 12.9 nmol/L) without a significant difference in vitamin B-6 intake. Hematocrit and hemoglobin were significantly higher in smokers (50± 3% and 167± 11 g/L, respectively) compared to non-smokers (48± 3% and 160± 9 g/L, respectively). The results of this study suggest that vitamin B-6 status of adult males in Riyadh is adequate with no urban vs. rural variation.

Evaluation of Vitamin B-6 Status of Saudi Adult Males In the Riyadh Region – Saudi Arabia

By

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APPROVED:

Major Professor, representing Nutrition and Food Management

Head of Department of Nutrition and Food Management

Dean of Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Abdullah Al-Assaf, Author

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CONTRIBUTION OF AUTHORS

Dr. James Leklem assisted in data collection in Riyadh – Saudi Arabia. Dr. Jim Ridlington and Karen Hardin were involved in laboratory analyses.

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EVALUATION OF VITAMIN B-6 STATUS OF SAUDI ADULT MALES IN THE RIYADH REGION – SAUDI ARABIA

INTRODUCTION

The kingdom of Saudi Arabia is one of the developing countries in the Middle East of Asia; Riyadh is its capital and largest city. The Riyadh region is composed of Riyadh city and many towns and villages surrounding the city. The latest statistical yearbook (1996) showed that the country has a population of 16,948,388; Saudi citizens comprise 72.6% of the total population. The number of Saudi males is 6,215,793, representing 50.5% of the Saudi population. The population of the Riyadh region is 3,834,986; Saudi citizens comprise 68.2% of this population. The number of Saudi males in the Riyadh region is 1,341,594.

The oil boom, which started in the mid-70s brought the country into the 20th century and caused a significant impact on all aspects of lifestyle, including food choices and nutritional habits. Recent nutritional studies have reported a high consumption of protein among Saudis. Al-Shoshan (1992) reported that there was a linear increase of total meat – fish, poultry and red meat – consumption in the eighties from 22 to 60 kg/capita/year. Miladi (1998) reported an increase in red meat consumption from 12.2 to 42.7 kg/capita/year, and an increase in milk consumption from 41.2 to 52.1 kg/capita/year for the period 1969-1994. These two studies, as well as most nutritional studies about Saudi Arabia, concluded that there is a prevalence of

adults being overweight and obese, as well as high consumption of protein and saturated fat, and recommended the establishment of programs to improve nutritional education and the awareness of healthy food selection. Among the nutritional data needed for Saudi population is the status of vitamin B-6 since its requirement is increased with an increase in the intake of dietary protein (Leklem, 1988b).

The term vitamin B-6 refers to the three primary forms of 3-hydroxy-5-hydroxymethyl-2-methylpyridine. These forms are pyridoxal (PL), pyridoxine (PN), and pyridoxamine (PM), as well as their phosphorylated forms. These three forms are converted in the liver to the physiological active form, pyridoxal-5′-phosphate (PLP) (Leklem, 1988a). PLP participates as a coenzyme in more than 100 enzymatic reactions; a majority are involved in amino acid metabolism. Thus, the requirement for vitamin B-6 increases with an increased intake of protein. A ratio of >0.016 mg vitamin B-6/g protein, is required for adequate status of the vitamin (Hansen et al, 1997). The requirement for vitamin B-6 may be increased in smokers compared to non-smokers, since previous data indicates that smokers have significantly lower plasma PLP concentration compared to non-smokers (Serfontein et al, 1986; Giraud et al, 1995).

Two studies have investigated the vitamin B-6 content of fourteen popular meat-based Saudi dishes, and six popular cereals and legume-based Saudi dishes and found that the vitamin B-6 content, compared to protein, was relatively low (Sawaya et al, 1986; Al-Jebrin et al, 1985). Since some studies have reported high protein consumption among Saudis and based on the last two mentioned studies, an

inadequate status of vitamin B-6 might be expected among the Saudi population. This inadequacy may be higher among rural compared to urban populations, based on the poor nutritional awareness and the low education level of rural compared to urban populations. However, to our knowledge no study has yet been done to investigate the requirements or status of vitamin B-6 in any group of the Saudi population.

Hypothesis:

The hypotheses tested in this study were:

1. Vitamin B-6 status, as measured by plasma B-6 vitamers, urinary 4-pyridoxic acid and dietary intake indexes in Saudi adult males is inadequate. This inadequacy is greater among rural populations compared to urban populations.
2. Plasma B-6 vitamers and urinary 4-PA levels are low in smokers compared to non-smokers.

Objectives:

The purpose of this study is to evaluate the vitamin B-6 status of both urban and rural populations of the Riyadh region – Saudi Arabia. Other specific objectives are:

1. Determine the intake of nutrients that can affect vitamin B-6 status, including dietary protein, dietary fiber (soluble and insoluble), riboflavin and zinc.
2. Determine the intake of macronutrients and selected micronutrients.

3. Compare the concentration of plasma vitamin B-6 vitamers and urinary 4-PA excretion between smokers and non-smokers.
4. Determine the vitamin B-6 content of some Saudi foods, as well as some common spices used in Saudi Arabia.

LITERATURE REVIEW

Vitamin B-6

History

For the past 70 years scientists have investigated vitamin B-6 and its physiologic functions. The vitamin and its name were first introduced by György in 1934 as an essential substance for growth in rats (György, 1934). In 1938, György and Lepkovsky, as well as three other groups, reported the isolation of pure crystalline vitamin B-6 (György, 1938; Lepkovsky, 1938). In 1939, the chemical structure of the vitamin was identified as 3-hydroxy-5-hydroxymethyl-2-methylpyridine, and the vitamin was named pyridoxine because of the similarity of its structure to pyridine (György and Eckhardt, 1939). In 1942, Snell and co-workers discovered the presence of other forms of the vitamin as a result of observances that natural materials were more active than pyridoxine in stimulating the growth of certain microorganisms. These other forms were formed from pyridoxine or its esters and were found to be pyridoxal and pyridoxamine (Snell et al, 1942). Two years later, Gunsalus et al, (1944) observed that in a low vitamin B-6 media, pyridoxal or pyridoxal and ATP restored low tyrosine decarboxylase activity of *S. fasecalis,* which indicated that pyridoxal phosphate is the co-enzyme form of vitamin B-6. The phosphorylation position of pyridoxal was determined by Heyl et al, (1951) and the co-enzyme form of the vitamin was determined to be pyridoxal-5′-phosphate (PLP). By the 1960s, it was known that

there were three interrelated forms of the vitamin: pyridoxine (PN), pyridoxal (PL) and pyridoxamine (PM), and all could be converted to the physiological functional form pyridoxal 5′-phosphate (György, 1971).

Structure and Chemistry

The term vitamin B-6 refers to all biologically active forms of the vitamin, which include three basic forms and their phosphorylated forms. The three basic forms are derivatives of 3-hydroxy-5-hydroxymethyl-2-methylpyridine, and they are PL, PN and PM. Their phosphorylated forms are pyridoxal 5′ phosphate (PLP), pyridoxine 5′ phosphate (PNP), and pyridoxamine 5′ phosphate (PMP). These forms are illustrated in Figure 1.1. An additional form of vitamin B-6 that is found only in plant foods is glycosylated pyridoxine [pyridoxine 5′ β-D-glucoside, (Fig. 1.2)], which is important in determining vitamin B-6 bioavailability.

B-6 vitamers are soluble in water and minimally soluble in organic solvents. PN, PL and PM are relatively heat-stable under acidic conditions, but heat-labile under alkaline conditions (Leklem, 2001). In aqueous solutions, PL, PN and PM are light sensitive at neutral or alkaline conditions, but relatively stable under acidic conditions (Ang, 1979). PLP is the physiologic active coenzyme form of vitamin B-6; it is covalently bound to enzymes by formation of a Schiff base with the ε-amino group of a lysine residue (Leussing, 1986).

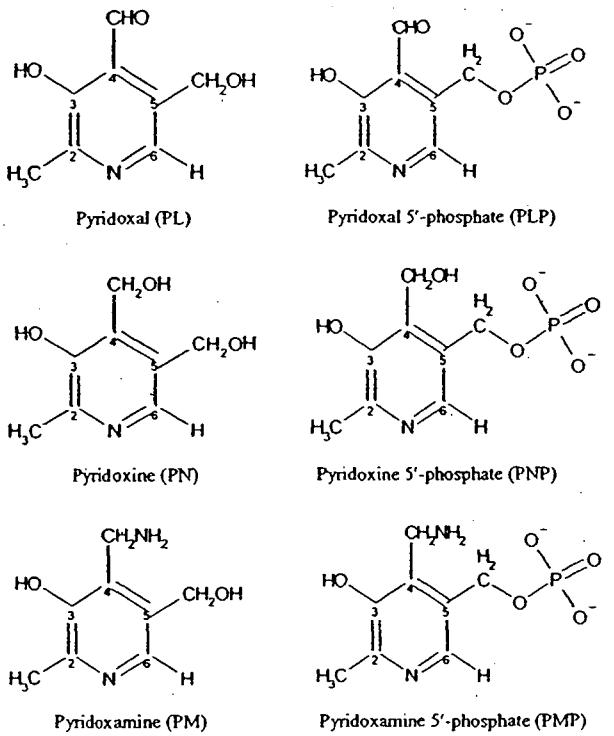


Fig. 1.1. Structure of the B-6 vitamers. (Adapated from Leklem, 2001).

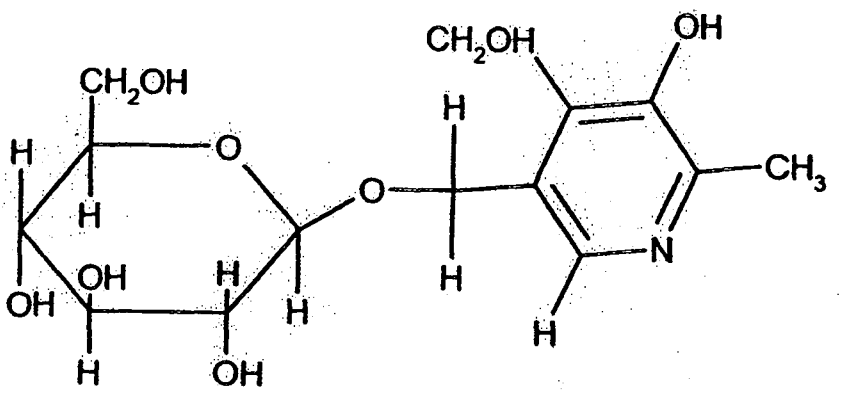
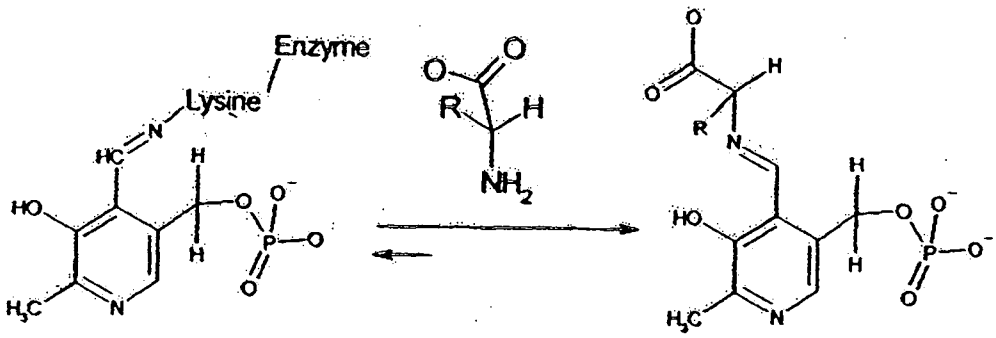


Fig. 1.2. Structure of pyridoxine 5′ β-glucoside (adapted from Leklem, 1988b).

Most PLP functions as a coenzyme are achieved by forming the Schiff base with the amino group of the substrate that is involved in the enzymatic reaction. Fig. 1.3 illustrates this formation.



PLP Bound to an enzyme as Schiff base Schiff base with an amino acid

Fig. 1.3. Schiff base formation between pyridoxal 5'- phosphate and an amino acid (adapted from Leklem, 2001).

Occurrence in foods

In general, PN and PM, or their phosphorylated forms, are the primary forms found in plant foods, whereas PLP is the primary form found in animal foods (Leklem, 1988b). As mentioned in the previous section, a glycosylated form of the vitamin, glycosylated pyridoxine, is also found in plant foods. Chicken, fish, beef and pork are major sources of the vitamin among animal foods, whereas bananas, avocados, legumes, walnuts and brown rice are the major sources of the vitamin among plant foods. Milk and dairy products, as well as certain fruits and vegetables, contain small

amounts of the vitamin. Vitamin B-6 and the percentage of the three basic form contents of selected foods are shown in Table 1.1. The content of the glycosylated form in selected foods is shown in Table 1.2. Other important sources of the vitamin are dietary supplements and fortified foods, such as cereals. Fortified cereals on average contain 1.5 mg B-6/100g. Both supplements and fortification use the pyridoxine form because of its stability.

Table 1.1
Vitamin B-6 content of selected foods and the percentage of the three basic forms.

Food	Vitamin B-6 (mg/100g)	PN (%)	PL (%)	PM (%)
Fruit				
Bananas, raw	0.510	61	10	29
Avocados, raw	0.420	56	29	15
Raisins, seedless	0.240	83	11	6
Apricots, dried	0.169	81	11	8
Apricots, raw	0.070	58	20	22
Oranges, raw	0.060	59	26	15
Apples, red delicious	0.030	61	31	8
Peaches, canned	0.019	61	30	9
Vegetables				
Spinach, raw	0.280	36	49	15
Potatoes, raw	0.250	68	18	14
Cauliflower, raw	0.210	16	79	5
Broccoli, raw	0.195	29	65	6
Corn, sweet	0.161	6	68	26
Cabbage, raw	0.160	61	31	8
Peas, green, raw	0.160	47	47	6
Carrots, raw	0.150	75	19	6
Beans, lima, frozen	0.150	45	30	25
Tomatoes, raw	0.100	38	29	33
Legumes and Nuts				
Soybeans, dry, raw	0.810	44	44	12
Walnuts	0.730	31	65	4
Lentils	0.600	69	13	18

Table 1.1 cont.

Beans, white, raw	0.560	62	20	18
Filberts	0.545	29	68	3
Peanut butter	0.330	74	9	17
Pecans	0.183	71	12	17
Almonds, without skin, shelled	0.100	52	28	20
Cereals and Grains				
Rice, brown	0.550	78	12	10
Wheat flour, whole	0.340	71	16	13
Wheat, cereal, flakes	0.292	79	11	10
Cornmeal, white and yellow	0.250	11	51	38
Barley, pearled	0.224	52	42	6
Rice, white, regular	0.170	64	19	17
Oatmeal, dry	0.140	12	49	39
Wheat flour, all-purpose, white	0.060	55	24	21
Meat, Poultry and Fish				
Chicken breast	0.683	7	74	19
Tuna, canned	0.425	19	69	12
Beef, raw	0.330	16	53	31
Pork, ham, canned	0.320	8	8	84
Salmon, canned	0.300	2	9	89
Sardines, Pacific, canned, oil	0.280	13	58	29
Flounder, fillet	0.170	7	71	22
Milk, Eggs and Cheese				
Egg, whole	0.110	0	85	15
Cheddar cheese	0.080	4	8	88
Milk, cow, homogenized	0.040	3	76	21
Milk, human	0.010	0	50	50

All values are taken from (Orr, 1969)

Table 1.2
Vitamin B-6 and glycosylated vitamin B-6 content of selected foods (adapted from Leklem, 2001)

Food	Vitamin B-6 (mg/100 g)	Glycosylated B-6 (mg/100g)
Fruits		
Bananas	0.313	0.010
Avocados	0.443	0.015

Table 1.2 cont.

Raisins, seedless	0.230	0.154
Apricots, dried	0.206	0.036
Tomato juice, canned	0.097	0.045
Orange juice, fresh	0.043	0.016
Blueberries, frozen	0.046	0.019
Pineapple, canned	0.079	0.017
Peaches, canned	0.009	0.002
Vegetables		
Potatoes, dried	0.884	0.286
Potatoes, cooked	0.394	0.165
Spinach, frozen	0.208	0.104
Cauliflower, frozen	0.084	0.069
Broccoli, frozen	0.119	0.078
Carrots, canned	0.064	0.055
Carrots, raw	0.170	0.087
Cabbage, raw	0.140	0.065
Beans and legumes		
Peanut butter	0.302	0.054
Lentils	0.289	0.134
Soybeans, cooked	0.627	0.357
Beans, navy, cooked	0.381	0.159
Beans, lima, frozen	0.106	0.039
Peas, frozen	0.122	0.180
Beans, garbanzo	0.653	0.111
Nuts/Seeds		
Filberts	0.587	0.026
Walnuts	0.535	0.038
Almonds	0.086	0.000
Sunflower seeds	0.997	0.355
Cereals and Grains		
Wheat bran	0.903	0.326
Rice, brown	0.237	0.055
Rice, bran	3.515	0.153
Rice, cereal, puffed	0.098	0.007
Rice, cereal, fortified	3.635	0.382
Animal products		
Tuna, canned	0.316	None detected
Chicken breast, raw	0.700	None detected
Beef, ground, cooked	0.263	None detected
Milk, skim	0.005	None detected

Absorption and Transport

Most studies investigating the absorption of vitamin B-6 were performed in animals; there are a limited number of such studies in humans. After ingestion, the non-phosphorylated forms of the vitamin (PN, PL and PM) are absorbed by a nonsaturable passive diffusion in the proximal jejunum. Absorption of the phosphorylated forms is very limited; they are dephosphorylated by intestinal phosphatase, mainly alkaline phosphatase and then are absorbed (Henderson, 1985; Middleton, 1990). Henderson (1985) reported that PM is absorbed at a slower rate than PL and PN. This study reported that when rats were fed ^{3}H-labeled B-6 vitamers, percentage of the labeled vitamers transported to the mucosa and perfusate of the rat intestine after 10 minutes were 23, 40, and 19 for PN, PL, and PM, respectively. This illustrated that PM is absorbed at a slower rate than PN and PL. In humans, similar findings were reported by Wozenski et al (1980) who found an increased elevation of plasma PLP and total B-6, as well as urinary B-6 excretion after consumption of PL and/or PN compared to PM.

Hydrolysis of PLP by alkaline phosphatase can be inhibited by the presence of two intraluminal constituents: ethanol and albumin (Middleton, 1986a and 1986b). Ethanol can inhibit the phosphatase in vitro, whereas albumin binds PLP via Schiff base reaction. The binding of PLP to albumin is pH dependent; it increased at high pH and is decreased at low pH, which suggests that absorption of PLP is significant in the upper small intestine. Since this binding occurs via a Schiff base, amino acids and

oligopeptides, which can also form a Schiff base with PLP, can also inhibit the hydrolysis of PLP (Middleton, 1990).

In the plasma PLP and PL comprise more than 90% of the vitamin B-6 (Leklem, 1996); PLP bound to albumin is the form released from the liver and then dephosphorylated to PL before transport across cell membrane because the phosphorylated form can not pass through cell membranes. The PLP and PL in plasma, as well as the PL in erythrocytes, are the major source of B-6 vitamers available to tissues. PN can also be taken up and transported by erythrocytes and converted to PLP or PL, which are bound to hemoglobin (Anderson et al, 1989).

Metabolism

Previous studies have reported that several organs are involved in the metabolism of B-6 vitamers. However, the liver is the major organ involved in vitamin B-6 metabolism, and the main source of plasma PLP (Lumeng et al, 1974; Lumeng et al, 1985; Leklem, 2001; Coburn, 1990; Coburn, 1996).

Following absorption, the unphosphorylated forms of the vitamin are taken up by the liver by passive diffusion and then converted to their phosphorylated forms within the cytoplasm of the hepatocyte. This process is catalyzed by pyridoxal kinase and requires both adenosine triphosphate (ATP) as a source of the phosphate group and zinc (Li et al, 1974). Both PNP and PMP are then converted to PLP via an oxidation process by a flavin-dependent enzyme known as PMP/PNP oxidase. These metabolic interconversions are shown in Figure 1.4.

Pyridoxal kinase is found in several tissues such as heart, brain, lung, kidney, pancrease, spleen, liver and muscle (Hanna et al, 1997); whereas a low activity of the oxidase is found in muscle and there is a relatively higher activity in erythrocytes and liver (Lumeng et al, 1985). Thus, the liver is responsible for converting dietary PN and PM to PNP and PMP and then to PLP; other tissues take up PLP (after dephosphorylation) from the circulation and take up PL and convert it to PLP (Merrill and Henderson, 1990; Leklem, 2001)

Another function of the liver is the irreversible conversion of PL, which comes from either the circulation or dephosphorylation of PLP, to 4-pyridoxic acid (4-PA). The 4-PA is then excreted in urine. This step is achieved by aldehyde oxidase, which requires flavin adenine dinucleotide (FAD) (Leklem, 1988a).

Merrill et al (1984) investigated the activities of the kinase, oxidase and phosphatase enzymes involved in vitamin B-6 metabolism in liver biopsy samples from five patients without hepatic disease. They reported that rate of PL phosphorylation was higher than rate of dephosphorylation, whereas the rate of PL oxidation to 4-PA was similar to rate of phosphorylation. The rates of PN and PM phosphorylation were lower than rates of conversion of PNP and PMP to PLP. Accumulation of PLP in the liver is regulated by these enzyme activities. The accumulated PLP inhibits PN and PM oxidase which decreases the conversion of PMP and PNP to PLP (Merrill et al, 1984; Leklem, 1996). Also, the accumulation of PLP increases the activity of phosphatase, which increases the conversion of PLP to PL and

subsequent conversion to 4-PA and excretion in the urine. This accumulation also enhances the release of PLP from the liver to circulation (Merrill et al. 1984).

The excretion rate of vitamin B-6 was studied in human subjects administered either PL, PM or PL (Wozenski et al, 1980). The study reported that rate of urinary 4-PA and B-6 was maximized in the first three hours after ingesting the doses, which suggests that oxidation from PL to 4-PA was favored. The study also confirmed that urinary 4-PA is the major form of excretion of vitamin B-6 and accounts for 40-60% of the daily intake.

Since pyridoxine 5′ phosphate oxidase requires riboflavin as FMN (flavin mononucleotide), riboflavin status is important in conversion of both PNP and PMP to PLP. Madigan et al (1998) reported that consumption of riboflavin supplements (25 mg/day) by elderly subjects increased their plasma PLP concentration. However, Lakshmi and Bamji (1974) reported that PLP levels in the blood of subjects with riboflavin deficiency were normal and consumption of riboflavin supplements did not affect these levels significantly. Another important nutrient in vitamin B-6 metabolism is zinc, since it is essential for alkaline phosphatase activity. Wan et al (1993) reported that zinc status, expressed as plasma alkaline phosphatase activity, affects vitamin B-6 status as reflected by the concentration of plasma PLP.

The PLP formed in the liver is either used in the liver as a coenzyme for many amino acid metabolic reactions or released to the circulation bound to albumin. PLP is bound to albumin in a molar ratio of 2:1 at neutral pH via a Schiff base reaction with high affinity (Lumeng et al, 1974). In human, fasting plasma PLP and PL comprise

75-90% of total vitamin B-6, with PLP consisting of 50-75% of the total B-6, pyridoxine is the next abundant form of the vitamin in the plasma (Coburn and Mahuren, 1983; Hansen et al, 2001). In addition, 4-PA, the end product of vitamin B-6 metabolism is also found in human plasma (Hansen et al, 2001). Similar distribution of vitamin B-6 forms in plasma was reported by Lumeng et al (1985) and Coburn and Mahuren (1983); 66% for PLP, 27% for PL, 0.9% for PMP, 2.3% for PM and 4.5% for PN and 52% for PLP, 21% for PL, 7.4% for PMP, 1.8% for PM and 17% for PN, respectively. Plasma PLP concentration is affected by pyridoxine supplementation. Lumeng et al (1974) reported that administration of a 25 mg pyridoxine supplement daily resulted in a four to five-fold increase in plasma PLP within four days. After discontinuing the supplementation, the concentration decreased by 4-6 μmol/L/day for five days, but was still above the value observed before consuming the supplement.

Erythrocytes also contain a relatively high concentration of PLP, PL and PN (Hansen et al, 2001; Anderson et al, 1989). PL and PN are taken up by a simple passive diffusion into erythrocytes and because erythrocytes contain both kinase and oxidase enzymes, both PL and PN can be converted to PLP (Mehansho and Henderson, 1980). PL in the erythrocyte is tightly bound to the alpha chain of hemoglobin, which increases the oxygen binding affinity of hemoglobin, whereas PLP is tightly bound to the beta chain of hemoglobin, which lowers the oxygen binding affinity of hemoglobin (Benesch et al, 1977; Maeda et al, 1976). Ink et al (1982) reported that PL accumulates rapidly in erythrocytes and this accumulation is independent of PLP concentration. They also reported that PL binding to hemoglobin

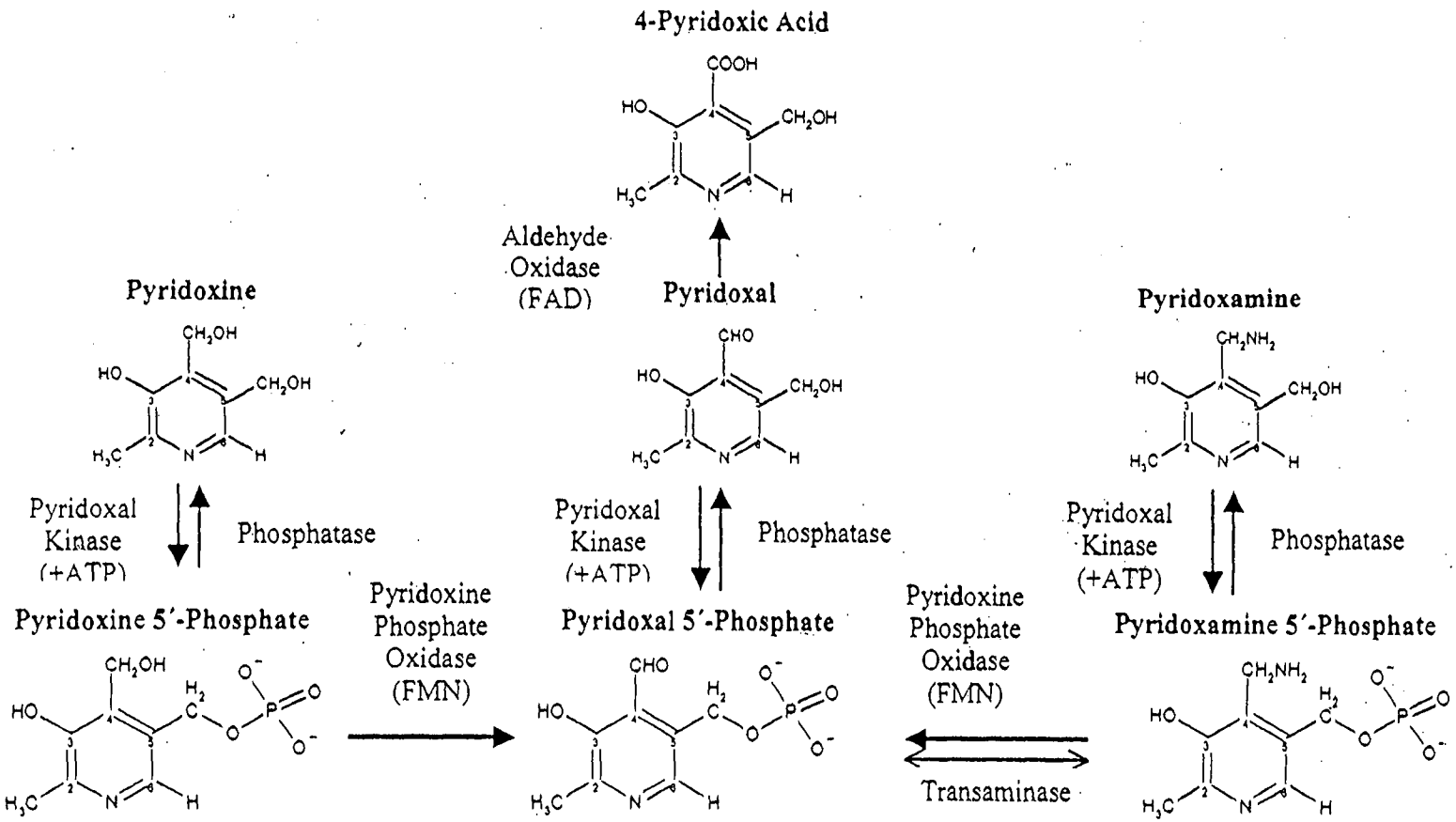


Fig. 1.4. Metabolic interconversion of the B-6 vitamers (adapted from Leklem, 1988b)

is stronger than its binding to albumin. This explains why erythrocyte PL concentration is four to five times greater than plasma PL concentration (Leklem, 1988a). An overview of the interorgan metabolism and transport of vitamin B-6 is shown in Figure 1.5.

Previous descriptions indicated that the liver is the major organ for processing the ingested vitamin B-6 and the organ that releases B-6 vitamers to other tissues via plasma. Both liver and erythrocytes play an important role in vitamin B-6 metabolism. However, under conditions of caloric deficit, muscles can release some of their PLP reservoirs to supply other tissues via the blood (Black et al, 1978). Muscles contain a high amount of vitamin B-6, compared to other organs, due to the large muscle mass. The vitamin B-6 content of body organs and blood is illustrated in Table 1.3.

A unique characteristic of muscle, besides being the major reservoir of vitamin B-6, is that muscle does not appear to be affected by B-6 deficiency, unlike other pools in the body. One of the early studies (Black et al, 1978) in animals reported that muscle PLP is bound to glycogen phosphorylase; both muscle phosphorylase and total

Table 1.3. Vitamin B-6 Content of Body Organs[1]

Organ	Amount (μmol)
Muscle	800-1000
Liver	18-24
Blood plasma	0.12-0.24
Erythrocyte	0.08-0.20

[1]Based on data from (Coburn. 1988) and (Leklem. 2001).

B-6 in muscle increased with increased intake of PN, but when the intake of PN was restricted, no change was observed. They also reported a positive correlation between B-6 content in muscle and the amount of phosphorylase, thus changes in the phosphorylase can be used as an indicator for the reservoir content. Another study in animal by Bode et al (1991) reported that decline of glycogen phosphorylase with aging was accompanied by a lowering of PLP content in muscle.

Studies in human subjects reported similar findings. Coburn et al (1991) fed young male subjects a diet low in vitamin B-6 (1.76 μmol/day) for six weeks and observe a slight non significant decreases in muscle B-6 content and glycogen phosphorylase (3.4% and 2.3%, respectively) from baseline, whereas PLP concentrations in plasma and erythrocyte, as well as urinary 4-PA excretion, were significantly lowered.

Bioavailability

Bioavailability of a nutrient refers to the portion of the ingested nutrient that is absorbed and metabolically utilized. Determination of vitamin B-6 bioavailability is important for determining both its requirement and status. Methods used to determine the bioavailability include balance studies, during which intake and excretion are determined, measuring an in vivo response after a defined level of deficiency has been reached, and examination levels of the nutrient or its metabolite in blood over a specified period of time after ingesting the nutrient (Leklem, 1988c; Leklem, 2001).

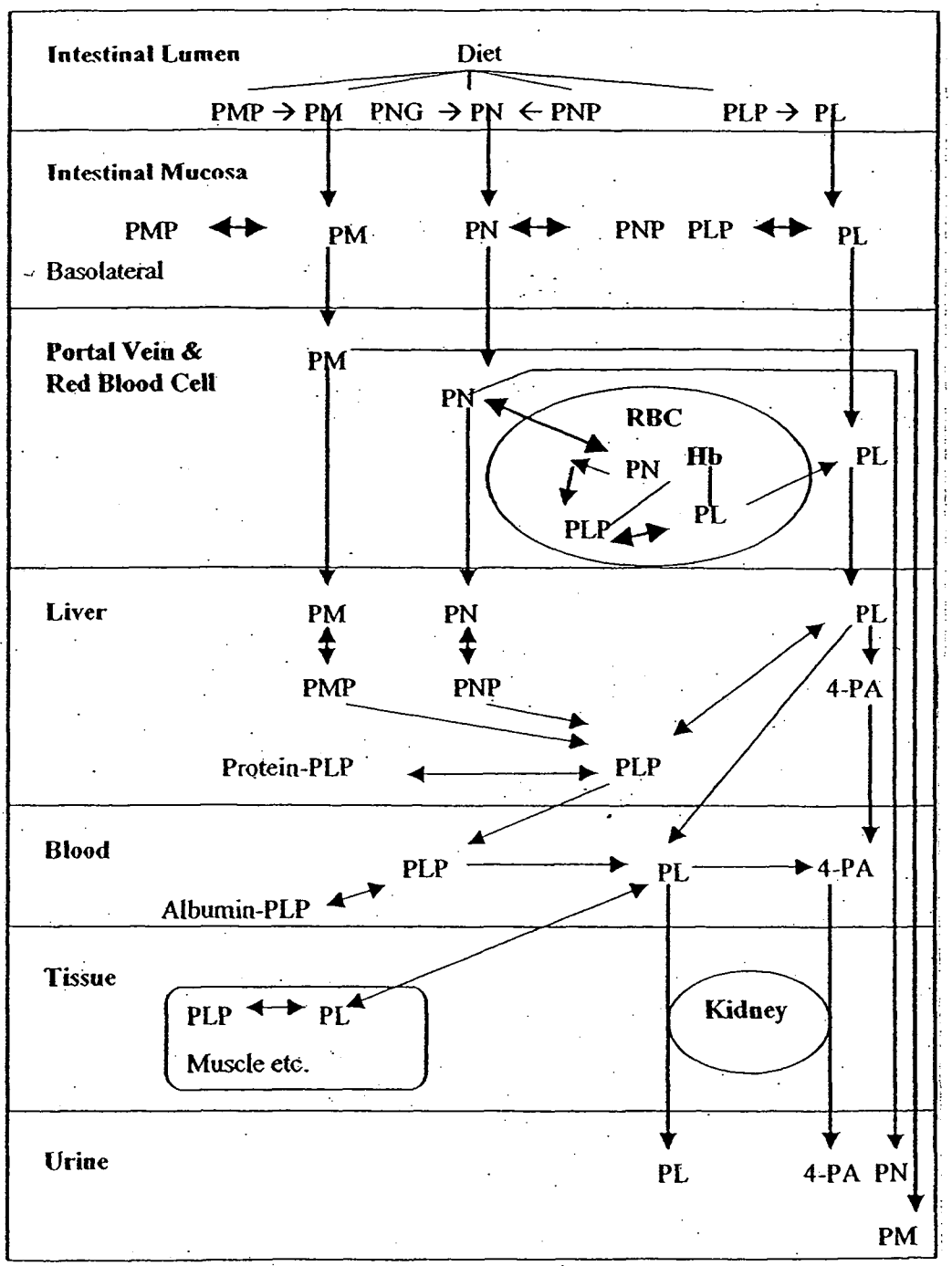


Fig. 1.5. Overview of vitamin B-6 transport, metabolism and excretion (adapted from Leklem, 1996)

The major factors affecting bioavailability of vitamin B-6 are the presence of conjugated pyridoxine glucoside and/ or the presence of dietary fiber type and amount (Leklem, 1988c; Reynolds, 1988; Hansen et al, 1996a; Leklem, 2001).

The glucoside form of vitamin B-6 is found only in plant foods, thus, previous studies considered that bioavailability of vitamin B-6 from animal food is quite high and could reach 100% (Reynolds, 1988). Total bioavailability of vitamin B-6 in a mixed diet is inversely related to the presence of the glycosylated form. The content of vitamin B-6 and the glycosylated form in selected foods is shown in Table 1.2. Bioavailability of the glycosylated form depends on hydrolysis of the β-glucosidic bond in order to release the pyridoxine.

To illustrate the mechanism of the antagonistic effect of the glycosylated form, Zhang et al (1993) studied isolated rat hypotocytes and found that pyridoxine-5-β-D-glucoside is transported into liver cells at about 20% of the rate of pyridoxine, and partially hydrolyzed. It also competitively inhibits the cellular uptake of pyridoxine. This study provided strong evidence that intact pyridoxine-5-β-D-glucoside inhibits the transport of pyridoxine and potentially inhibits the transport of pyridoxal and pyridoxamine into cells by competing for the transport mechanism. However, a well-defined mechanism for that antagonistic effect has not yet been established. Maeno et al (1997) examined the bioavailability of pyridoxine and pyridoxine derivatives, pyridoxine-α-D-glucoside (synthetic form) and pyridoxine-β-D-glucoside in a long-term feeding experiment with rats. Bioavailability was evaluated by plasma and red blood cell PLP, liver kynureninase activity and urinary excretion of xanthurenic acid

and 4-PA. The study reported an average bioavailability of 22, 84, 100% for pyridoxine-β-D-glucoside, pyridoxine-α-D-glucoside and pyridoxine, respectively. The glycosylated form of the vitamin also inhibited the utilization of pyridoxine in addition to not being totally bioavailable. Recently, Mackey et al (2002) purified and investigated lactase phlorizin hydrolase from rat small intestine mucosa, which hydrolyzes the β-glucosidic bond of pyridoxine glucoside and found that the enzyme was inhibited by glucose, lactose, and cellubiose, but was not inhibited by pyridoxine. Moreover, a reaction mixture containing the glycosylated form with lactose and lactase phlorizin hydrolase enzyme resulted in formation of a pyridoxine derivative known as pyridoxine disaccharide. This derivative has not been reported previously. This suggests that there may be an adverse effect of lactose on the hydrolysis of the glycosylated form of vitamin B-6 that would in turn lower its bioavailability. Armada et al (2002) reported that the activity of lactase phlorizin hydrolase in the brush border and the activity of cytosolic pyridoxine-5-β-glucoside hydrolase, which also hydrolyzes the glucosidic bond, declined with age in rats.

In a human study, Hansen el al (1996a) fed female subjects two diets containing either a high or low amount of pyridoxine glucoside for a period of eighteen days. The high diet contained 1.52 mg/day, and the low diet contained 1.44 mg/day of vitamin B-6, of which 27% and 9% was pyridoxine glucoside, respectively. Feeding the high pyridoxine glucoside diet, compared to the low diet, resulted in lower urinary 4-PA and total vitamin B-6 excretion, lower plasma total B-6, and red blood cell PLP concentration and higher fecal content of total B-6, which indicated that the

pyridoxine glucoside form was not totally bioavailable. The study reported that reduced bioavailability of pyridoxine glucoside was equal to a loss of 15-18% of the total vitamin B-6 intake. Another human study by Nakano et al (1997) investigated the effect of pyridoxine glucoside on vitamin B-6 bioavailability in healthy human subjects that were administered an oral dose of either $^{2}H_2$ pyridoxine or $^{2}H_2$ pyridoxine glucoside. The study measured the urinary excretion of labeled 4-PA and found that bioavailability of pyridoxine glucoside was about a 50% relative to pyridoxine. In a second trial, a nonlabeled pyridoxine glucoside was administered and compared to the labeled pyridoxine, the excretion of $^{2}H_2$ 4-PA derived from labeled pyridoxine was inversely related to the proportion of the ingested nonlabeled pyridoxine glucoside. This suggests that the glycosylated form of the vitamin may affect pyridoxine metabolism. Ferroli and Trumbo (1994) studied dependence of bioavailability on age and reported that vitamin B-6 bioavailability does not depend on age. They administered ^{2}H pyridoxine to two groups of healthy men aged 20-30 and 60-70 years. Both were fed a controlled diet. No significant differences were found between the two groups for plasma PLP concentration and urinary excretion of total or ^{2}H 4-PA, which suggested that bioavailability of the vitamin is not altered by aging.

Dietary fiber is considered another major factor that affects the bioavailability of vitamin B-6. Hudson et al (1988) investigated the bioavailability of vitamin B-6 from rat diets containing wheat bran, cellulose, or free-form fiber. Bioavailability was determined based on growth, urinary 4-PA excretion and ^{14}C pyridoxine turnover in the liver, with increasing dietary B-6 intake. There were no significant differences in

these indices, which suggested that dietary fiber, as cellulose or the indigestible component of wheat bran, did not adversely affect the bioavailability of vitamin B-6. In human study, Leklem et al (1980) investigated the bioavailability of vitamin B-6 from three types of bread in human subjects. Each subject consumed one of the three types of bread: whole wheat, white, and white enriched with vitamin B-6. Consumption of whole wheat bread, compared to the other types, resulted in higher fecal vitamin B-6 excretion and lower urinary 4-PA excretion, and a lower level of plasma PLP only, compared to white enriched bread. These findings suggested that vitamin B-6 was less available from whole wheat bread compared to white bread and/ or enriched bread. A similar study fed 10 male subjects a constant diet that contained 1.7 mg of vitamin B-6 /day for a period of 18 days, with and without the addition of 15 g of cooked wheat bran (Lindberg et al, 1983). The presence of the cooked wheat bran in the diet caused an increase in fecal vitamin B-6 excretion and a decrease in urinary 4-PA excretion, suggesting that wheat bran decreased the bioavailability of vitamin B-6. Another study compared vitamin B-6 bioavailability from whole wheat bread, peanut butter and tuna (Kabir et al, 1983). In this study, eight men were fed a diet containing 1.6 mg/day vitamin B-6, with 50% coming from one of the three experimental foods and 50% from a basal diet. Bioavailability was determined based on urinary 4-PA and total B-6 excretion, fecal B-6 excretion and plasma PLP concentration. The study found that urinary 4-PA and vitamin B-6 excretion was higher in the tuna period, compared to that in the other two periods. Fecal vitamin B-6 excretion was lower when tuna was fed compared to that when peanut butter was fed.

The study concluded that bioavailability of vitamin B-6 from whole wheat bread and peanut butter relative to tuna was 75% and 63%, respectively. While the whole wheat bread diet contained more dietary fiber (35g) than the peanut butter diet (24g), vitamin B-6 in whole wheat bread was relatively more available. Even though the difference was not statistically significant, it seemed that the relatively higher dietary fiber content in the whole wheat bread did not adversely affect the bioavailability of vitamin B-6. The difference may be explained by other factors, which include the presence of the glycosylated form and the effect of food processing. In another human metabolic study, Kies et al (1984) investigated the effect of three bran (wheat, corn and rice) supplements (20g of each type per subject per day) on the availability of vitamin B-6. These bran supplements (wheat, corn and rice) contained 0.26, 0.35 and 0.48 mg of vitamin B-6, respectively. The bran supplements were added to a controlled diet that contained 1.24 mg of vitamin B-6. The study reported that supplementation the diets with brans increased the total intake of vitamin B-6, however it reduced total and free vitamin B-6 loss in urine which suggested that these bran supplements reduced the bioavailability of vitamin B-6. Moreover, the brans had an adverse effect on the vitamin availability from other foods. The study reported that there are several explanations for this observation; the most plausible one is the action of fiber, which include decreases the fecal transit time and shortens the time for absorption. In addition, dilution of gastrointestinal tract contents and increased of surface areas for absorption could lead to excretion of nutrients.

Most studies examining the effect of dietary fiber on the bioavailability of vitamin B-6 used rice bran, corn, or whole wheat bread, foods that contain more insoluble dietary fiber than soluble dietary fiber. To examine the effect of insoluble vs. soluble dietary fiber, Nguyen et al (1981) examined the effect of cellulose (insoluble), pectin (soluble) and bran composed of 60% pectin and 40% insoluble fiber (30% cellulose and 10% lignin) on the bioavailability of vitamin B-6 in rats and chicks. Each diet contained 5% of one of the previous fibers and two levels of pyridoxine, 0.5 and 1.0 μg/g. The bioavailability was determined based on the indices of growth, feed consumed, feed efficiency, liver PLP and aspartate aminotransferase activity. The results showed that in the chick, bran and cellulose had little effect, and no effect of pectin on the bioavailability of the vitamin. In rats, results were inconclusive, but in general, suggested there was no adverse effect of the three fibers on the bioavailability of the vitamin. This inconsistency may have been due to using the rat as an animal model because in rats, fiber-dependent alterations in the intestinal synthesis of vitamin B-6 and probable utilization of the synthesized vitamin may have complicated the evaluation of dietary B-6 bioavailability.

Recently, Roth-Maier and co-workers published a series of papers, which evaluated the bioavailability of nutrients using a precaecal digestibility technique. This technique uses ileo-rectal anasstomosis, which bypasses the colon and lets the digesta move from the ileum directly to the rectum. This technique eliminates any vitamin synthesized by intestinal microflora. These nutrients studied were thiamine, riboflavin, pantothenic acid (Roth-Maier et al, 1996); niacin (Roth-Maier et al, 2000) and vitamin

B-6 (Roth-Maier et al, 2002). This last study used the domestic pig as an animal model because it has been validated as a valuable model for studying digestive physiology in humans. Results showed that on average, digestibility of vitamin B-6 from plant foods was 10% lower, compared to animal foods. The results are shown in Table 1.4.

Table 1.4

Precaecal digestibility (%) of B-6 vitamers from different food sources[1]

Food source	PN	PL	PM
Egg, boiled	ND	82 ± 3	27 ± 6
White cabbage	98 ± 1	69 ± 2	76 ± 2
Bananas	95 ± 1	ND	91 ± 1
Corn	14 ± 8	79 ± 5	64 ± 4
Fish, stewed	22 ± 31	89 ± 1	85 ±2
Milk powder	85 ± 17	87 ± 5	75 ± 5
Brown rice, boiled	43 ± 13	ND	ND
Soybeans, boiled	98 ± 2	46 ± 8	32 ± 16
Barley	91 ± 3	ND	36 ± 14
Wheat bran	69 ± 2	ND	26 ± 21
Brewer's yeast, dried	78 ± 8	ND	80 ± 5
Rye	71 ± 2	41 ± 21	29 ± 21
Soybean meal	85 ± 1	82 ± 11	57 ± 10

[1]: Data are means ± SD. ND: Intake of B-6 vitamers was below detection limit.

Biochemical Functions

Among the B-complex vitamins, and perhaps other vitamins, vitamin B-6 is unique because of its numerous functions. This comes from its ability as PLP to

participate in Schiff base formation with substrates that contain nitrogen. The one exception is the interaction of PLP with the enzyme glycogen phosphorylase. Table 1.5 summarizes the cellular process systems affected by PLP and Figure 1.6 illustrates the overall metabolism pathways that require vitamin B-6 as PLP.

Table 1.5
Cellular process systems affected by PLP (adapted from Leklem, 2001).

Cellular process or enzyme	Function/system influenced
One-carbon metabolism, hormone modulation	Immune function
Glycogen phosphorylase, transamination	Gluconeogenesis
Tryptophan metabolism	Niacin formation
Heme synthesis, transamination, O_2 affinity	Red cell metabolism and formation
Neurotransmitter synthesis, lipid metabolism	Nervous system
Hormone modulation, binding of PLP to lysine on hormone receptor	Hormone modulation

Gluconeogenesis

Gluconeogenesis is a process, which synthesizes glucose form precursors including lactate, alanine, glycerol and pyruvate. Glucose is considered a major source of fuel to the body during short fasting or starvation states. In gluconeogenesis, PLP participates as a coenzyme in transamination reactions for alanine aminotransferase, which converts alanine to pyruvate. Moreover, PLP participates in glycogen

degradation as a coenzyme for glycogen phosphorylase, which releases glucose-6-phosphate from the glycogen chain. In skeletal muscle, alanine coming from gluconeogenic amino acids and pyruvate, as well as lactate that come from anaerobic glycolysis, are transported to the liver and converted to glucose via gluconeogenesis. This glucose is transported back to skeletal muscle to serve as a source of energy. This cycle is known as Cori cycle and is illustrated in Figure 1.7.

In an animal study, Angel (1980) observed that vitamin B-6 deficiency caused a decrease in blood glucose due to limited formation of pyruvate from alanine. However, the same trend was not observed in humans (Rose et al, 1975). In this study female subjects were fed a diet containing 0.19 mg of vitamin B-6 for 4 weeks. No significant change in fasting blood glucose was observed.

The relation between vitamin B-6 and diabetes was investigated in diabetic rats by Nair et al (1998). This study measured blood glucose level and kinetic parameters of glutamate dehydrogenase in control (non diabetic rats) and four groups of diabetic rats that were insulin treated, pyridoxine treated (100mg/Kg body weight), insulin and pyridoxine treated and untreated. The study found that treatment with insulin and pyridoxine compared to treatment with insulin or pyridoxine alone was better in lowering the increased maximal velocity of glutamate dehydrogenase to the control state as well as lowering blood glucose level. This study suggested that a combined administration of insulin and pyridoxine served as a better control for diabetes. In a human study, Hollenbeck et al (1983) investigated the relation between diabetes and vitamin B-6 in six insulin-dependent diabetic women. In a cross-over design, the

subjects consumed experimental diet (65% carbohydrates and 20% fat) and control diet (45% carbohydrates and 40% fat). The mean intake of vitamin B-6 during the experimental and control diets feeding periods was 2.3± 0.4 mg/day and 1.8± 0.3 mg/day, respectively. Whole blood vitamin B-6, plasma vitamin B-6 and plasma PLP concentration were below lower limits of normal values. This study concluded that diabetic subjects had a lower mean plasma PLP concentration than that of controls and thus, may have poor vitamin B-6 status.

Niacin Formation

Niacin is one of the essential B-complex vitamins. Tryptophan, which is an essential amino acid, can be converted to niacin. One of the steps in this synthetic pathway involves the enzyme kynureniase, which requires PLP as a coenzyme. Leklem et al (1975) reported that vitamin B-6 deficient subjects (they consumed 0.19 mg B-6 /day for four weeks) excreted significantly lower amounts of niacin metabolites than subjects receiving 2.0 mg of vitamin B-6/day. This study suggested that vitamin B-6 deficiency had a negative effect on formation of niacin from tryptophan.

Immune Function

PLP is a coenzyme for serine transhydroxymethylase, which is one of the important enzymes involved in one-carbon metabolism. Changes in one-carbon

metabolism can affect nucleic acid synthesis, which can impact immune function (Schirch and Mason, 1963). Chandra et al (1981) examined the effect of pyridoxine deficiency on weights and number of lymphocytes of thymus and spleen in rats. The study compared a pyridoxine deficiency group to a pair-fed control group and a group of animals that were fed ad libitum. The pyridoxine deficient group compared to the other two groups had a significant reduction in both weights and number of lymphocytes in the thymus and spleen. These results demonstrated that pyridoxine was important for optimal cell-mediated immune responses. In another study by Ha et al (1984), the effect of various levels of vitamin B-6 on immune responses of T cells in mice was examined. Mice were fed diets contained 7, 1, 0.1 or 0 mg of pyridoxine /Kg diet, which represented 700, 100, 10 and 0% of requirement, respectively. The study found that mice, which were fed 0.1 or 0 mg/Kg diet pyridoxine compared to those, that were fed 7 or 1 mg/Kg diet pyridoxine had significantly reduced primary T cell-mediated cytotoxicity (T-CMC). The mice that were fed 0 mg/Kg diet pyridoxine had significantly reduced secondary T-CMC. These results suggested that the primary T-CMC was dose dependent whereas the secondary T-CMC was more resistant to pyridoxine deficiency. This study concluded that vitamin B-6 deficiency differentially affected functional subpopulations of lymphocytes. In another animal study, Inubushi et al (2000) found that in extreme vitamin B-6 deficient mice, both ovalbumin-dependent antibody production and alanine aminotransferase activity in the liver significantly decreased. These metabolic changes were expressed more strongly with a high protein diet compared to a low protein diet. This was explained by the higher

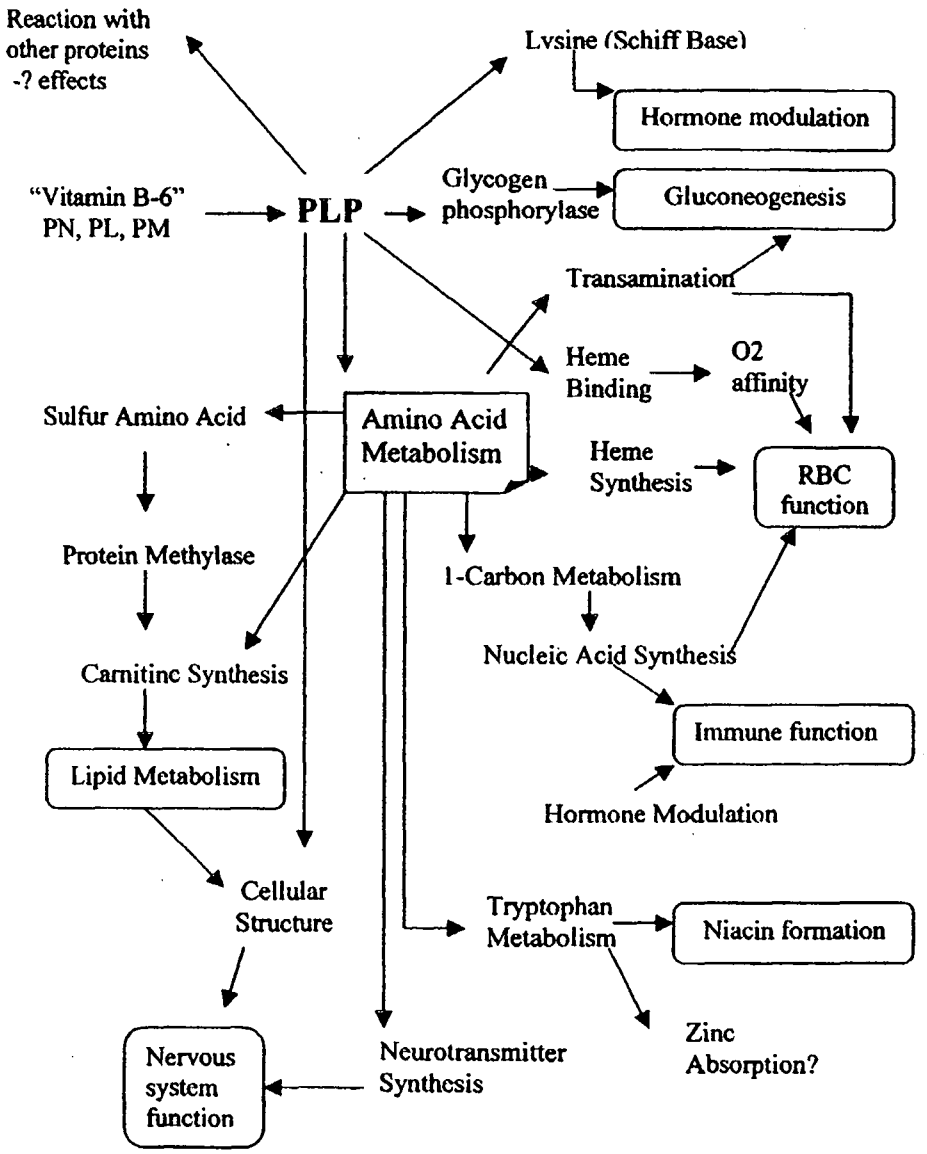


Fig. 1.6. Metabolic pathways that require vitamin B-6 (adapted from Leklem, 1988a)

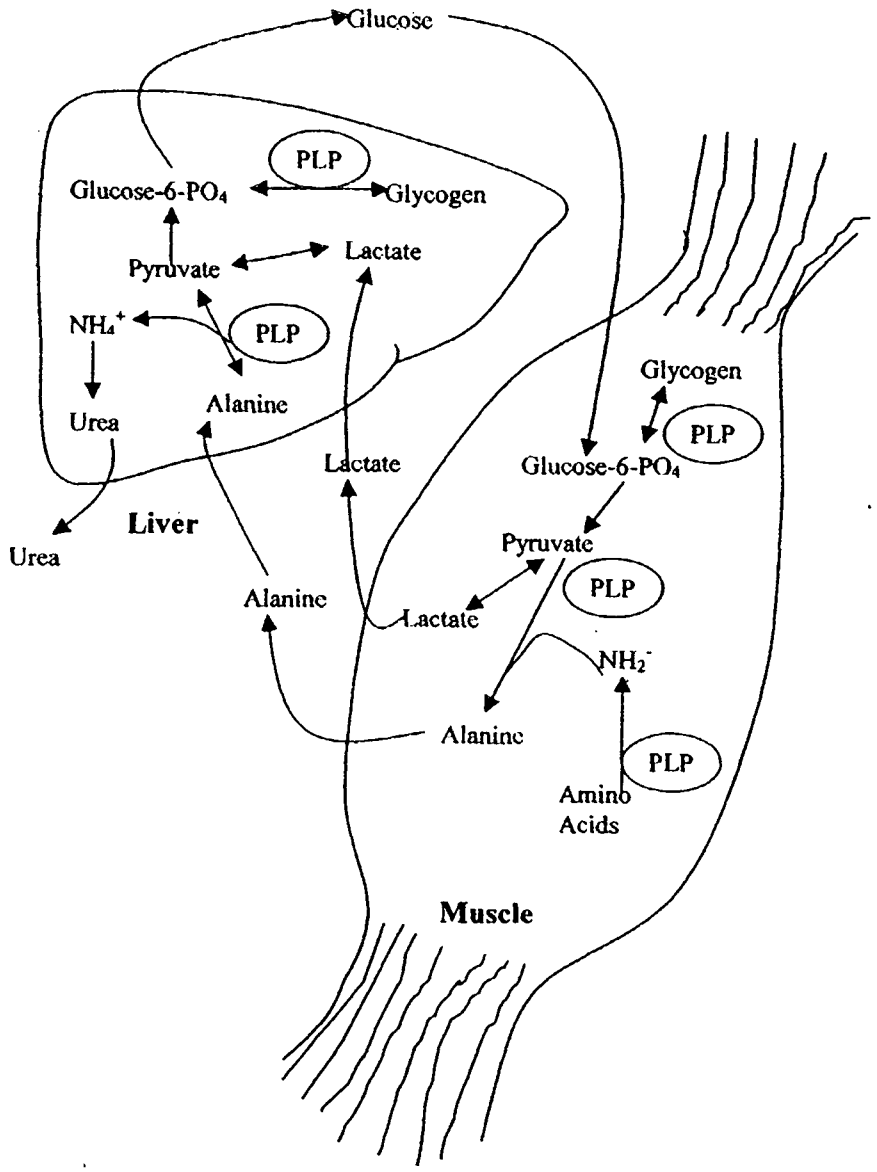


Fig. 1.7. Cori-alanine cycle, role of PLP in glucose and alanine metabolism. (adapted from Leklem, 1985)

demand for PLP for amino acid metabolism enzymes in the case of the high protein diet. A human study by Talbott et al (1987) reported that ingestion of 50 mg pyridoxine for eight weeks resulted in improved lymphocyte function in 11 elderly females whose immune response was impaired. A controlled human study by Van den Berg et al (1988) examined the effect of marginal vitamin B-6 deficiency on immune responses. This study compared two groups that were receiving the same diet. One of these two groups (control group) received vitamin and mineral supplements whereas the other group (treatment group) received the same supplement but without pyridoxine. The treatment group had a low intake of vitamin B-6, which induced marginal vitamin B-6 deficiency. Compared to the control group, the treatment group had a significantly lower percentage of helper T cells as well as a lower concentration of serum immunoglobin. In another human study Meydani et al (1991), reported that a diet-induced vitamin B-6 deficiency significantly decreased the percentage and total number of lymphocytes. Repletion with 2.9 and 1.9 mg/day of pyridoxine for males and females, respectively, returned the response to baseline values. A recent study by Kwak et al (2002) examined the effect of increased intake of vitamin B-6 on lymphocyte proliferation in seven young women. Results showed that consumption of 2.1 mg of vitamin B-6/day compared to 1.5 mg/day for seven days significantly increased lymphocyte proliferation. The study also reported that lymphocyte proliferation was significantly correlated with vitamin B-6 intake. Results of this study illustrated an improvement in vitamin B-6 status with intake higher than the recommendation of the DRI (Kwak et al, 2002).

Nervous system

Vitamin B-6 plays an important role in the nervous system because PLP serves as a coenzyme in several neurotransmitter syntheses, such as taurine, dopamine, histamine, serotonine and norepinephrine (Dakshinamurti, 1982). PLP is required for the enzyme glutamic acid decarboxylase, which catalyzes synthesis of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) from glutamic acid. In an in vitro study, Denner and Wu. (1985) examined the effect of PLP concentration on regulation of GABA synthesis in rat brains by examining the activity of glutamate decarboxylase in absence and presence of exogenous PLP (0.2mM). This study found that absence of PLP decreased the activity of glutamate decarboxylase and the synthesis of GABA. The study concluded that PLP has an important role in regulation of GABA function. Serotonin and histamine are synthesized by decarboxylation of tryptophan and L-histidine by the PLP dependent enzymes 5-hydroxytryptophan decarboxylase and L-histidine decarboxylase, respectively. Lee et al (1988) found that when histidine and tryptophan are consumed above the recommended level, consumption of vitamin B-6 supplements resulted in a significant increase in histamine and serotonin concentrations in rat brain. On the other hand, Schaeffer et al (1998) fed rats an excess amount of pyridoxine (700 mg/Kg diet) for 10 weeks and found an increase in amino acids and neurotransmitters concentration in brain regions, as well as an increased concentration of serine, glycine and aspartate in serum. In addition, an excess intake of pyridoxine (700 mg/Kg diet) changed the binding properties of serotonin receptors in

the brain cortex without changing its concentration. McCarty (2000) reported that in animals, a pyridoxine deficiency resulted in an increase in hypertension and decreased central production of serotonin and GABA. On the other hand, supplementation with pyridoxine lowered hypertension and increased the production of the two neurotransmitters, serotonine and GABA. The investigator suggested that high does of pyridoxine in humans might be useful in treatment of hypertension. Vasdev et al (2002) reported two mechanisms that may explain the effect of vitamin B-6 on hypertension in rats and human. The first mechanism is increasing the production of cysteine from methionine; because two enzymes in the synthesis pathway require PLP (cystathionine ß-synthase and cystathionase). Increased production of cysteine will increase the excretion of excess metabolic aldehydes, normalizing vascular calcium channels and thus, lower blood pressure. The second mechanism is lowering aldehydes production via improvement of glucose metabolism.

Hormone Modulation

PLP acts as a modulator of steroid hormone action. In an in vitro study Litwack et al (1985) have found that PLP forms a Schiff base with a lysine residue on the steroid receptors, which results in inhibition of the binding of the steroid complex to DNA. Compton and Cidlowski (1986) showed that PLP binds at two sites; a steroid binding site and a DNA binding domain on the receptor. They also reported that alteration in the steroid receptor – DNA complex binding resulted in decreased action

of the steroid. An explanation for this effect was reported by Bunce and Vessal (1987). They investigated the effect of vitamin B-6 and zinc deficiency on estrogen uptake and retention in the uterus. They found that number of estrogen receptors was not affected by the deficiency, whereas the uptake of estrogen was significantly increased in both vitamin B-6 and zinc-deficient animals, which implies there was an increase in the sensitivity to the hormone. In a similar manner, Allgood and Cidlowski (1992) reported that vitamin B-6 modulates the transcriptional activation of other steroid hormone receptors, including androgen, progesterone, and estrogen receptors.

Erythrocyte Formation and Function

PLP is essential for erythrocyte formation because it is required by the enzyme δ-aminolevulinic acid synthase, which catalyzes the condensation of glycine and succinyl-CoA to form δ-aminolevulinic acid, a compound that is a precursor for heme synthesis (Kikuchi et al, 1958). Thus, vitamin B-6 has an important role in erythropoiesis and a deficiency in the vitamin can lead to hypochromic anemia (Horrigan and Harris, 1968). PLP also serves as a coenzyme for alanine and aspartic transaminases in the erythrocyte. The activity of these transaminases is used to determine the status of the vitamin. As mentioned in the metabolism section, PLP and PL affect the oxygen binding affinity of hemoglobin in the erythrocyte. This oxygen binding affinity, associated with PLP and PL bound to heme, may be important in sickle-cell anemia (Reynolds and Natta, 1985).

Lipid Metabolism

The exact role of PLP in lipid metabolism is still unclear. In animal studies, Sabo et al (1971) reported that vitamin B-6 deficiency in rats resulted in an increase in triglyceride synthesis, but Angel and Song (1973) reported that a deficiency of vitamin B-6 resulted in decreased triglyceride synthesis. A third study by Angel (1975) showed no significant difference in triglyceride synthesis between vitamins B-6 depleted rats and controls. The effect of vitamin B-6 on fatty acid metabolism is also unclear. Cunnane et al (1984) found that a vitamin B-6 deficiency in rats resulted in increased phospholipid levels of linoleic acid and decreased levels of arachidonic acid in plasma, liver, thymus and skin. Another study by Tsuge et al (2000) reported that vitamin B-6 deficiency in rats impaired the metabolism of ω-3 polyunsaturated fatty acids from α-linoleic acid to eicosapentaenoic acid and docosahexaenoic acid. In contrast, Kim et al (1997) reported that young women who consumed a low vitamin B-6 diet (0.93 mg/day) for three weeks, did not show a significant difference in plasma fatty acid profile, compared to those who consumed a higher vitamin B-6 diet (2.6 mg/day). Vitamin B-6 also has a role in carnitine synthesis. Carnitine is an essential nitrogen compound that carries fatty acyl units to the mitochondria for β-oxidation and is synthesized from lysine and methionine. The initial reaction in the synthesis is catalyzed by lysine methyl transferase; the activity of this enzyme is decreased in vitamin B-6 deficient rats (Loo and Smith, 1986). In a rat study, Cho and Leklem (1990) found a significant decrease in total and free carnitine in the plasma,

heart, urine and skeletal muscle of rats fed a vitamin B-6 deficient diet for six weeks. Levels of carnitine were restored to control levels after rats were fed a vitamin B-6 replete diet for two weeks. Results of this study illustrated that vitamin B-6 is required in carnitine synthesis.

Homocysteine Metabolism

Homocysteine is a sulfur-containing amino acid that is an intermediary product in methionine metabolism. It is formed from the demethylation of methionine and follows one of two metabolic pathways; either remethylation to methionine or transsulfuration to cystathionine by the PLP-dependent enzyme cystathionine β-synthase. Recently, several studies reported the association between elevation of homocysteine in the blood and cardiovascular disease. People with high homocysteine levels have an approximately two-fold increase in risk of coronary disease, compared to those with low levels; this risk is similar to the risks associated with smoking or hypercholesterolemia (Fairfield and Fletcher, 2002; Fletcher et al, 2002). The role of vitamin B-6 in lowering homocysteine has been investigated in several studies. Siri et al (1998) studied the relation between hyperhomocysteinemia and plasma PLP concentration in 131 patients with severe coronary arthrosclerosis, and 88 subjects with minor or no coronary stenosis. They found that patients and healthy subjects in the upper quartile of plasma PLP concentration had significantly lower plasma homocysteine levels than those in the lower quartile. Similarly, Bates et al (1999)

observed that British men and women aged 65 years and over, participating in a National Diet and Nutrition Survey, had an inverse correlation between plasma PLP concentration and plasma homocysteine concentration, which enhances the hypothesis that vitamin B-6 may lower plasma homocysteine. McKinley et al (2001) reported that even low-dose supplementation of vitamin B-6 (1.6 mg/day) for 12 weeks for healthy elderly subjects aged 63-80 years who were folate and riboflavin-replete, effectively lowered fasting plasma total homocysteine. A recent study reported that supplementation of folic acid and vitamin B-6 (9 mg/day) for elderly coronary atherosclerosis patients for three weeks, resulted in a significant reduction in fasting plasma total homocysteine, as well as a reduction in plasma lipid profile (Mark et al, 2002.)

Assessment of Status

Determination of vitamin B-6 status is important in determining the metabolism of the vitamin, as well as the impact of excess or deficiency on human physiology. Leklem (1990) reviewed and classified methods of vitamin B-6 status assessment into three classes: direct, indirect and dietary methods. Table 1.6 lists these methods, as well as the minimum values indicating adequate status. Direct methods include measuring the actual B-6 vitamers in biological samples such as plasma, erythrocytes, and urine, whereas indirect methods measure metabolites produced by PLP-dependent enzyme activity pathways or the activity of these enzymes. There is no

one single index adequate to evaluate the status of the vitamin; a combination of indices is found to be the most reliable approach. Leklem (1990) suggested the use of at least three indices, including plasma PLP, urinary 4-PA, and an indirect measure, such as erythrocyte transaminase activity/ stimulation. Reynolds (1995) reviewed methods of vitamin B-6 status assessment and suggested the use of several indices. The review recommended a priority sequence of indices, which were plasma PLP coupled with plasma alkaline phosphatase activity, erythrocyte PLP, dietary intake of vitamin B-6 and protein, urinary excretion of 4-PA/creatinine and the activation coefficient of erythrocyte aspartate aminotransferase.

A. Direct Measurements

Direct measures include measures of B-6 vitamers or 4-PA in plasma, erythrocyte and urine, since tissue samples are not usually available. Plasma PLP is the most relevant indicator for vitamin B-6 status, but as mentioned previously, it cannot be used alone to reflect the vitamin status because it can be influenced by factors other than vitamin B-6 intake (Powers, 1999). Lumeng et al (1985) examined the relation between plasma PLP and levels of PLP in animal tissues. Plasma PLP was found to reflect the tissue content of PLP and thus plasma PLP was considered to be good indicator of the vitamin status. Shultz and Leklem (1981) found a significant relationship between vitamin B-6 intake and plasma PLP in humans. The study also found significant correlation between urinary 4-PA as well as urinary vitamin B-6

with vitamin B-6 intake. This study suggested that vitamin B-6 intake should be combined with dietary protein intake. Plasma PLP concentration was found more related to this combination compared to urinary 4-PA and urinary vitamin B-6. This study suggested that plasma PLP is the best single indicator of the vitamin status. Several other studies have also reported an increase in plasma PLP concentration with an increased intake of vitamin B-6 (Brown et al, 1975; Lee and Leklem, 1985; Leklem, 1990; Huang et al, 1998) and a decrease in plasma PLP with the decreased intake of vitamin B-6 (Kretsch et al, 1991; Meydani et al, 1991).

The concentration of PL in plasma is fairly constant which makes plasma PL a weak indicator of status, since it does not reflect intake (Reynolds, 1995). The reason for this constancy is that PL can rapidly diffuse across cell membranes and tends to reach the equilibrium state with the intracellular concentrations of PL (Reynolds, 1995). The only exception is during pregnancy where plasma PL concentration is significantly higher in pregnant women compared with same age non-pregnant women (Barnard et al, 1987).

The end product of B-6 metabolism is 4-PA, which is also considered a direct measure of the vitamin status. Urinary 4-PA changes rapidly in response to changes in vitamin B-6 intake, and is considered a short-term indicator of the vitamin status (Leklem, 1990). Under normal conditions, 40-60% of the daily intake of vitamin B-6 is excreted as 4-PA in urine (Shultz and Leklem, 1981). To assess the status of vitamin B-6 by urinary 4-PA, a 24-hour urine collection is required. Once 4-PA is in urine, it is

relatively stable. However, urine should be kept in a cool, dark, dry place to avoid any degradation of 4-PA that may lead to underestimating its concentration.

In addition, erythrocyte PLP levels have also been used as a direct measure to evaluate vitamin B-6 status. However, previous studies reported that it is a less sensitive response to change in status compared to plasma PLP (Kant et al, 1988; Hansen et al, 1997). However, PLP in red blood cells differ from PLP in plasma in influence of alkaline phosphatase. Since alkaline phosphatase exists on the outer membrane of red blood cell, its effect on PLP inside the cell is negligible (Reynolds, 1995).

At present, the most frequent direct indices that are used to evaluate vitamin B-6 status are plasma PLP concentration and urinary 4-PA excretion. Their cut off value of adequate status (Table 1.6) was reported by Leklem (1990). These cut off values were based on concentrations of PLP as responded to controlled intake of vitamin B-6 in both males and females. Powers (1999) reported that plasma PLP concentration in humans above 25 nmol/L could be achieved at intakes of vitamin B-6 above 1.0 mg/day. Thus, with the recommendation of the DRI for adult male (1.3 mg/day), the plasma PLP concentration should be >30 nmol/L. However, plasma PLP does not immediately reflect the intake as urinary 4-PA (Leklem, 1990). Thus, combination of these two direct measures is frequently used.

Plasma PLP is influenced by several factors, (Table 1.7). One of the important factors is age, which has been reported to have a negative correlation with plasma PLP, as well as alanine aminotransferase activity (Rose et al, 1976; Vanderjagt and

Garry, 1985; Hamfelt and Soderhjelm, 1988). Possible reasons for this include changes in dietary habits that resulted in lower intake of vitamin B-6, poor health status and alteration in vitamin B-6 metabolism.

Table 1.6
Methods for assessing vitamin B-6 status and suggested values for adequate status (adapted from Leklem, 2001).

Index	Suggested value for adequate status
Direct	
Blood	
Plasma pyridoxal-5-phosphate	>30 nmol/L[a]
Plasma pyridoxal	NV
Plasma total vitamin B-6	>40 nmol/L
Erythrocyte pyridoxal-5-phosphate	NV
Urine	
4-pyridoxic acid	>3.0 μmol/day
Total vitamin B-6	>0.5 μmol/day
Indirect	
Blood	
Erythrocyte alanine aminotransferase	>1.25[b]
Erythrocyte aspartate aminotransferase	>1.80[b]
Urine	
2g tryptophan load test: xanthurenic acid	<65 μmol/day
3g methionine load test: cystathionine	<351 μmol/day
Oxalate excretion	NV
Dietary Intake	
Vitamin B-6 intake, weekly average	>1.2-1.5 mg/day
Vitamin B-6: protein ratio	>0.02
Pyridoxine-β-glucoside	NV
Other	
Electroencephalogram pattern	NV

a: Values are dependent on sex, age and protein intake
b: For each aminotransferase measure, the activity coefficient represents the ratio of the activity with added PLP to the activity without PLP added.
NV: No value established.

In a human study by Lee and Leklem (1985), young women were compared to older women in their responses to a constant diet that contained 2.3-2.4 mg B-6/ day followed by the same diet supplemented with 8.0 mg pyridoxine. The study found that older women compared to young women had significantly lower concentrations of plasma B-6 and PLP as well as lower excretion of urinary vitamin B-6 and slightly higher urinary 4-PA with the constant diet. After supplementation, the only difference remained significant was urinary total B-6. Haller (1999) reviewed vitamin B-6 status in an elderly population in Europe and reported that mean plasma PLP levels were below 10 nmol/L in seven different geographic locations. The review reported that vitamin B-6 deficiency was one of the two most prevalent deficiencies among an elderly European population, particularly in central Greece and Italy. Similarly, Bates et al (1999) reported that plasma PLP concentration and 4-PA excretion in elderly British men and women was negatively correlated with age and positively correlated with vitamin B-6 intake. Moreover, the study suggested that plasma PLP and 4-PA were not ideal indicators for vitamin B-6 status among the elderly because plasma PLP was sensitive to metabolic conditions associated with inflammation and the acute phase reaction, while plasma 4-PA was sensitive to renal function. A human study by Reynolds et al (1988) found that vitamin B-6 status, as determined by plasma PLP and total vitamin B-6, was lower in elderly men compared to younger men. The study reported that another possible reason for this observation, in addition to that mentioned in Vanderjagt's study, was the slight elevation in alkaline phosphatase activity, which occurs with aging. This increased activity of alkaline phosphatase increases the rate of

hydrolysis of plasma PLP to PL. The consistency of results of the studies mentioned above illustrated that requirement of vitamin B-6 is increased with aging.

Another important factor is smoking, which was reported in several studies as having a negative correlation with plasma PLP. In an observational study, Serfontein et al (1986) reported that plasma PLP levels among 106 tobacco-smoking men were significantly lower than in 143 non-smoking men. Also, Ritchie and Singkamani (1986) measured plasma PLP in free living premenopausal women, including 12 smokers and 24 non-smokers, and found that the mean plasma PLP level was 29% lower for smokers, compared to non-smokers; the study did not report vitamin B-6 intake for the subjects. Giraud et al (1995) compared plasma B-6 vitamers, as well as erythrocyte PLP and PL between long-term tobacco smokers, chewers and non-users with non-significant different in vitamin B-6 intake. The study found a significant higher plasma PLP in non-users, compared to smokers and chewers. Plasma PL, PN, PM and 4-PA, as well as erythrocyte B-6 vitamers of the three groups were not significantly different. Similar results were reported by Serfontein and Ubbink (1988), who examined 103 sedentary adult smokers and 122 sedentary adult non-smokers, and found a significant lower (29%) concentration of plasma PLP in smokers, compared to that of non-smokers. In an elderly population, Pessah-Rasmussen et al (1990) examined plasma PLP concentrations in 95 male smokers and 63 male non-smokers and found that the mean plasma PLP level was 22% lower for smokers compared to non-smokers. The difference in vitamin B-6 status between smokers and non-smokers in free-living adult males was investigated by Giraud and Driskell (1994), daily

vitamin B-6 intake was 1.9 mg for smokers and 3.0 mg for non-smokers. The results showed that there was no significant difference in plasma PLP, plasma 4-PA and urinary 4-PA concentrations between smokers and non-smokers. Explanation of these results came from the difference in the intake between the two groups, even though was not significant. A controlled metabolic study by Sindihebura-Ruhumba (1999) investigated vitamin B-6 status for five smokers (4 males and 1 female) and four non-smokers (3 males and 1 female). The investigator fed the subjects a constant diet that provided 1.95 mg and 1.65 mg of vitamin B-6 for males and females, respectively. For the last 10 days, subjects consumed an additional 2 mg pyridoxine –as a supplement-. Vitamin B-6 indices were measured during the two periods (before and after consuming the supplements). Results showed that during the two periods, mean concentration of plasma PLP, 4-PA and red blood cells PLP were significantly lower among smokers compared to non-smokers. Consumption of supplements resulted in a 85.8% increase of plasma PLP concentration for smokers, but it was 48.5% lower than that of non-smokers. Urinary 4-PA excretion did not differ significantly between the two groups throughout the 20 days. However, mean values of 4-PA excretion were consistently higher in non-smokers compared to smokers. Results of this study illustrated that there was an adverse effect of smoking on vitamin B-6 metabolism.

Another factor that can influence plasma PLP concentration is physical activity. Leklem and Shultz (1983) investigated plasma PLP concentrations before and after 4500-m run in adolescent trained athlete males. The investigators reported a significant increase in plasma PLP concentration after the run. Another study by

Manore et al (1987) examined the effect of exercise on plasma PLP concentration in trained and untrained female subjects.

Table 1.7
Factors influencing plasma PLP concentration (adapted from Leklem, 1999)

Factor	Effect on plasma PLP
Diet	
↑ vitamin B-6	Increase
↑ protein	Decrease
↑ glucose, acute	Decrease
↓ Bioavailability	Decrease
Physiologic	
↑ exercise, aerobic	Increase
↑ age	Decrease
Pregnancy	Decrease
↑ Alkaline phosphatase activity	Decrease
Smoking, chronic	Decrease

The effect was examined in four types of diets: moderate and high carbohydrate diets that contained 2.3 and 2.4 mg of vitamin B-6, respectively, and two other diets that were supplemented with pyridoxine (8.0 mg/day). Results showed that plasma PLP concentration increased with exercise in all diets. This result indicated that exercise significantly change vitamin B-6 metabolism; this change was not influenced by level of carbohydrate. Leonard and Leklem (2000) investigated the effect of extreme exercise (50-km ultramarathon) on plasma PLP concentration and found that prolonged exercise decreased plasma PLP concentration. Results of the above two studies illustrate that exercise increase plasma PLP concentration at the beginning, but when the exercise is prolonged, the concentration of plasma PLP is decreased.

B. Indirect Measurements

The term "indirect" comes from the fact that these measures reflect either the levels of metabolites that are influenced by levels of intracellular PLP or measure activities of enzymes that are PLP dependent. There are two PLP-dependent metabolic pathways that are sensitive to vitamin B-6 deficiency: metabolism of tryptophan and methionine. In addition, measuring two erythrocyte transaminases is also considered an indirect measure of vitamin B-6 status. These two transaminases are erythrocyte aspartate transaminase (EAST) and erythrocyte alanine transaminase (EALT).

Brown (1985) reported that vitamin B-6 status can be measured following a 2 g of L-tryptophan load – or 50 mg/Kg body weight– by measuring the urinary excretion of xanthurenic acid. Xanthurenic acid excretion is a sensitive index of vitamin B-6 deficiency and correlated with other indices of vitamin B-6 status such as urinary 4-PA and plasma PLP (Brown, 1985). However, the tryptophan load test can't be used with subjects having diseases because tryptophan pyrrolase, which convert tryptophan to kynurenine, may be induced by the stress of the disease. Thus, changes in metabolites may be due to disease rather than vitamin B-6 deficiency. Brown (1988) indicated that the sensitivity of this method is influenced by liver disease and some types of cancers. In addition to the this limitation, Brown (1985) reported that premenopausal women using oral contraceptives and post-menopausal women using estradiol had an abnormal tryptophan load tests, which suggests that estrogens alter the activity of kynureninase, and thus simulate a functional deficiency of vitamin B-6.

However, Leklem (1990) indicated that under conditions where factors known to affect tryptophan load test are absent, the test remains to be a valid indicator of vitamin B-6 status, but he also indicated that the test has been used less frequently after the advent of direct measurements.

A second indirect method is the measurement of urinary cystathionine after a 3 g-methionine load. Linkswiler (1981) reported that in young men and women, who were deprived of vitamin B-6 for seven days, the amount of excreted cystathionine was elevated and this elevation continued as the length of deficiency increased. However, Reynolds (1995) reported that this method is less specific and less used due to the multitude of conditions that can elevate plasma homocysteine because methionine is first converted to homocysteine and then homocysteine is converted to cystathionine. In addition, as in tryptophan load test, level of dietary protein has an influence on methionine metabolism (Leklem, 1990). Both the tryptophan and methionine load tests are sensitive indicators of vitamin B-6 deficiency and are reflective of the vitamin status in the liver. However, their use has mainly been limited to vitamin B-6 depletion and repletion studies, which make their use in status assessment in need of further evaluation (Leklem and Reynolds, 1981).

The third indirect method is the measurement of erythrocyte aminotransferase activity and/ or stimulation. This method measures the basal activity of two erythrocyte enzymes that require PLP for their activity. These two enzymes are aspartate aminotransferase (EAST) and alanine aminotransferase (EALT). An alternative method is to measure their basal activity and measure their stimulation in

the presence of excess PLP (increase in activity). This later method is known as the activation coefficient and expressed as the ratio of the enzyme activity in presence of exogenous PLP divided by enzyme activity without PLP (Reynolds, 1995). In a human study, Brown et al (1975) found that EALT was more responsive to vitamin B-6 intake than EAST. In this study women were fed a deficient diet of vitamin B-6 (0.19 mg) for four weeks. They reported a significant reduction in basal EAST and EALT activity. The EAST activity changed significantly in the fourth week of deficiency and did not respond significantly to supplementation of vitamin B-6 (1.84 mg) whereas EALT activity was more responsive. This is in agreement with Leklem (1990) who concluded that EALT is a better indicator of vitamin B-6 status compared to EAST. Reynolds (1995) indicated that there are two limitations for the use of erythrocyte transaminases; the basal amount of both EAST and EALT is affected by number of clinical conditions, thus a change in their activity can be due to reasons other than vitamin B-6 status. He also indicated that analysis of frozen samples yield different values for both basal activities and activation coefficients of EAST and EALT. Also, the natural presence of hemoglobin in erythrocyte suspensions has been reported to alter aminotransferase activities. Because the activities of these enzymes change slowly, their measurements are considered a long-term indicators of vitamin B-6 status and reflect long-term vitamin B-6 intake (Leklem, 1990; Reynolds, 1995). However, because the turnover of red blood cells and the unclear relationship between enzymes activity and other measures of vitamin B-6 status, use of this method is not

recommended as a primary indicator of the vitamin status (Leklem and Reynolds, 1981).

C. Dietary Methods

Assessment of vitamin B-6 status by dietary methods includes measuring the intake of vitamin B-6, the glucoside form of the vitamin and vitamin B-6 to dietary protein ratio (Table 1.6). These measures depend on two factors; determining the content of vitamin B-6 in foods and determining the actual intake of foods. Determining the content of vitamin B-6 in foods was first developed by Atkin et al in 1943 by a microbiological method. Even though this method has been refined, it is analytically reliable and accurate and the source of most of the available data of the vitamin content in foods (Leklem, 2001). On the other hand, Trumbo (1995) suggested that a HPLC method is more preferable since it can separate and quantify both phosphorylated and conjugated forms of the vitamin. Including the conjugated form of the vitamin in the intake will result in overestimation of the available amount of the vitamin that is consumed by an individual. Reynolds (1995) reported that disadvantages of using dietary methods include determining the actual amount of food intake and the variation of the conjugated form concentrations in foods. An important factor in using vitamin B-6 intake as an index of the vitamin status is considering the intake of dietary protein. This was shown in a human study by Miller et al (1985); this study indicated that plasma concentrations of total B-6 and PLP in males who consumed constant amount of vitamin B-6 were inversely proportional to the amount

of protein in the diet. The interrelationship between dietary protein and vitamin B-6 will be discussed in the requirement section. Shultz and Leklem (1981) reported that there was a significant correlation of both female and male plasma PLP, urinary 4-PA and urinary vitamin B-6 levels with both dietary vitamin B-6 and vitamin B-6: protein ratios. Thus, these biochemical measures reflect subject's recent intake of vitamin B-6. However, as mentioned before, dietary index should be combined with other indices in evaluation the status of vitamin B-6 (Leklem, 1990; Reynolds, 1995).

Requirements

Considering the numerous functions of vitamin B-6, understanding the requirement for this vitamin becomes very important. Since the major function of vitamin B-6 is in amino acids metabolism, several studies suggested that the vitamin requirement should be based on protein intake. Factors other than dietary protein that may contribute to the requirement of vitamin B-6 are listed in Table 1.8.

One of the early studies by Baker et al (1964) suggested that vitamin B-6 requirement for adult men with high protein intake (100 gm) was 1.75-2.0 mg/day whereas for those with low protein intake (30 gm) the requirement was 1.25-1.5 mg/day. This study indicated that vitamin B-6 requirement should be related to level of dietary protein intake. Similar findings were reported in another human study by Miller and Linkswiler (1967). This study showed that feeding a diet containing 100 gm/day of protein required 1.5 mg/day of pyridoxine whereas a similar diet containing

33 g/day of protein required 1.25 mg/day of pyridoxine. These requirements were determined based on the tryptophan load test. Miller at al (1985) investigated the effect of various levels of dietary proteins (0.5, 1.0, and 2.0 grams of protein/kg body weight, with a constant intake of vitamin B-6 of 1.6 mg/day) on the metabolism of the vitamin in eight adult males. The results showed that with an increased intake of protein, the body retained vitamin B-6; this retention was shown by a negative correlation between protein intake and the excretion of urinary 4-PA and concentration of plasma B-6. The study suggested that the body retained the vitamin in order to meet the demand for amino acid catabolism. Because of the significant relation between level of dietary protein and concentration of plasma and urinary excretion of B-6 vitamers found in this study, this study concluded that protein intake must be considered in evaluating the requirement of vitamin B-6. Similarly, Hansen et al (1996b) fed nine women diets that provided various amounts of protein, 0.5, 1.0, and 2.0 g protein/kg body weight, with a constant intake of B-6 (1.25 mg/day). The study reported a significant negative correlation between increased protein intake and urinary 4-PA excretion, as well as plasma PLP concentration. Additionally, increased protein intake was positively correlated with erythrocyte alanine aminotransferase percentage stimulation and urinary post-tryptophan load excretion of xanthurenic acid. These results illustrated the increased requirement of B-6 with increased intake of dietary protein. This study found that a vitamin B-6 to protein ratio of 0.020 mg/g was inadequate to achieve plasma PLP concentration above 30 nmol/L in 66.7% of participants. The study suggested that a ratio of > 0.020 mg B-6/g protein is needed

for adequate vitamin B-6 status. Another study investigated vitamin B-6 requirement based on a constant protein intake and varying intake levels of vitamin B-6; Hansen et al (1997) fed young women a constant protein diet (85 g/day), and four levels of vitamin B-6 (1.03, 1.33, 1.73, and 2.39 mg/day). Vitamin B-6 status indicators, which included urinary 4-PA and total B-6 excretion and plasma PLP concentration, were significantly different among the various intakes of the vitamin. The study suggested that a ratio of vitamin B-6 to protein of >0.016 mg/g was necessary to maintain adequate status of the vitamin. Similarly, Huang et al (1998) fed young women a constant high protein diet, which provided 1.55g/kg body weight with increased vitamin B-6 to protein ratios, which were 0.005, 0.013, 0.016, 0.017 and 0.021 mg/g. The study reported that a ratio of 0.019 mg vitamin B-g/g protein was required to normalize urinary 4-PA, plasma PLP, erythrocyte PL and PLP and erythrocyte alanine and aspartate aminotransferase activities. The study suggested that the RDA for vitamin B-6 based on a B-6 to protein ratio of 0.016 is not adequate. The increased requirements of vitamin B-6 due to the high protein intake is explained by stimulation of amino acid metabolizing enzyme activities in response to the high protein intake (Okada et al, 1998). A recent investigation of vitamin B-6 requirement by Hansen et al (2001) examined direct and indirect vitamin B-6 status indicators of healthy young women who consumed a controlled diet providing 1.2 g protein/kg body weight and 1.0 mg B-6/day as adjustment period, and then consumed diets that provided three different levels of vitamin B-6, which were 1.5, 2.1, and 2.7 mg. Plasma PLP and total B-6, urinary 4-PA and total B-6 and erythrocyte PL and PLP were positively

correlated with the increased intake of vitamin B-6. Results of this study suggested a requirement of 1.5 mg B-6 or a ratio of 0.020 mg B-6/g protein. The study also reported that in combination with other data, an intake of 1.7 mg/day of vitamin B-6 or a ratio of 0.018 mg B-6/g protein is required.

The quality of protein may also be important in determining the requirement of vitamin B-6. In an animal study by Fisher et al (1984) rats were fed either a high- or low-quality protein diet at two levels of vitamin B-6, which were 0.2 and 7.0 mg/kg diet. Results showed an insignificant reduction in urinary 4-PA, plasma PLP and total vitamin B-6 in the liver at the two levels of intake in the low-quality protein group, compared to the high-quality protein group. In another study, Sampson et al (1995) reported that feeding rats high-quality protein, as casein, compared to a low-quality protein diet such as wheat gluten with various levels of vitamin B-6 (0.0, 0.5, 1.0 and 1.5 mg/kg diet), did not affect the status indicators of the vitamin. In a human study, Hudson et al (1989) fed young women either an animal protein diet – mainly dairy and poultry products – or a plant protein diet –mainly various types of bran – with increasing intakes of vitamin B-6 of 0.5, 1.0, 1.5 and 2.0 mg/day. All diets provided 1.55 g protein/kg body weight. The study showed that animal protein digestibility was significantly higher than plant protein digestibility; the higher digestibility was not significantly affected by vitamin B-6 intake. Moreover, the nitrogen balance for both groups was not influenced by vitamin B-6 intake. These results suggested that short-term low intake of the vitamin does not affect protein utilization, as measured by protein digestibility and nitrogen balance. Kretsch et al (1995) fed healthy young

women either a high- or low-quality protein diet with increasing vitamin B-6 intake (0.5, 1.0, 1.5 and 2.0 mg/day) for three weeks. The low-quality protein was from plant sources, whereas the high quality protein was from animal sources.

Table 1.8
Factors affecting vitamin B-6 requirement (adapted from Leklem, 2001)

1. Dietary
   - Physical structure of a food.
   - Forms of vitamin B-6 natural; those due to processing.
   - Binding of forms of vitamin B-6.
2. Defect in delivery to tissues
   - Impaired gastrointestinal absorption.
   - Impaired transport – albumin synthesis and binding, phosphatase activity.
3. Physiological/biochemical
   - Physical activity – increased loss, glucogenesis
   - Protein – enzyme induction
   - Increased catabolism/turnover – phosphatase activity, illness
   - Impaired phosphorylation and/or interconversion, competing pathways, nutrient deficiencies, drugs
   - Pregnancy – demand of fetus
   - Growth – increased cell mass, repair
   - Lactation – adequate levels in milk
   - Excretion rate – urinary, sweat, menstrual loss
   - Sex – differences in metabolism
   - Age – differences in metabolism
4. Genetic
   - Apoenzyme defects – altered binding to apoenzyme
   - Altered apoenzyme levels – biochemical individuality
5. Disease prevention/treatment
   - Which? Heart, cancer, diabetes, PMS, kidney, alcohol

The results showed that the plant protein diet, compared to animal protein diet, did not affect vitamin B-6 requirements.

The 1989 RDA for vitamin B-6 for adult men and women aged ≥ 25y was 2.0 mg/day and 1.6 mg/day, respectively. This recommendation were based on average intake of dietary protein of 126 g and 100 g for men and women, respectively and a ratio of 0.016 mg B-6/g dietary protein. Studies mentioned above in this section illustrated the need of increasing the requirement of vitamin B-6 and to use a ratio > 0.016. Moreover, several studies mentioned in the biochemical functions section, especially in the immune function of vitamin B-6, reported the benefits of increasing the requirement of vitamin B-6. However in 1998, the Institute of Medicine lowered the RDA for vitamin B-6 to 1.3 mg/day for adult male and female (Table 1.9). This recommendation was calculated by using an Estimated Average Requirement (EAR) of 1.1 mg/day + 2 coefficient of variation of 10 percent. Even though several studies mentioned at the beginning of this section indicated that dietary protein must be considered in setting the requirement of vitamin B-6, the DRI used plasma PLP of at least 20 nmol/L as the criterion for establishment the EAR for vitamin B-6. Plasma PLP was used because it was considered the best single indicator of the vitamin status since it reflects tissue concentration.

Table 1.9
Dietary Reference Intake of vitamin B-6 for various groups

Group	Vitamin B-6 (mg/day)
Infants	
0-6 mo	0.1
7-12 mo	0.3
Children	
1-3 y	0.5
4-8 y	0.6
Males	
9-13 y	1.0
14-18 y	1.3
19-30 y	1.3
31-50 y	1.3
51-70 y	1.7
> 70 y	1.7
Females	
9-13 y	1.0
14-18 y	1.2
19-30 y	1.3
31-50 y	1.3
51-70 y	1.5
> 70 y	1.5
Pregnancy	
≤ 18 y	1.9
19-30 y	1.9
31-50 y	1.9
Lactation	
≤ 18 y	2.0
19-30 y	2.0
31-50 y	2.0

Source : Institute of Medicine, 1998.

Nutritional Studies in Saudi Arabia

The country of Saudi Arabia is among the richest and highest per capita income countries of the world. This high income combined with food affluence and lack of nutritional awareness has led to a state of overnutrition among the population. Generally, nutritional studies in Saudi Arabia are very limited. Of these studies, most agreed there has been a lack of nutritional education. Several studies reported the prevalence of obesity, diabetes mellitus and hypercholesterolemia among adult population as well as anemia among children (Sebai, 1988; Madani et al, 2000). There are no studies of vitamin B-6 status; however, the vitamin B-6 content of some popular Saudi foods has been determined. Sawaya et al (1986) reported the protein and vitamin B-6 content of fourteen meat-based popular Saudi dishes (Table 1.10). Al-Jebrin et al (1985) reported the protein and vitamin B-6 content of six cereals and legume-based popular Saudi dishes (Table 1.11). The two studies reported that selection of the dishes was based on their popularity and high frequency of intake.

Table 1.10
Protein and vitamin B-6 content of fourteen popular meat-based Saudi dishes

Dish name	Protein (g/100g)	Pyridoxine (B-6) (mg/100g)	B-6:Protein Ratio[a]
Margook (wheat dough stew)	5.1	0.068	0.014
Kabseh ma'dajaj (rice with chicken)	4.1	0.074	0.018
Kabseh ma'lahm (rice with lamb)	5.2	0.076	0.015
Kabseh ma'samak (rice with fish)	4.4	0.044	0.010

Table 1.10 cont.

Dajaj saleeg (rice with chicken and milk)	3.9	0.040	0.010
Sakshuka (omelet)	7.1	0.081	0.011
Mahshee Edams (stuffed vegetables)	3.6	0.073	0.020
Motabak ma'moz (banana pasta)	5.7	0.156	0.027
Motabaka ma'lahm (meat pasta)	9.4	0.072	0.007
Mataziz (Saudi pizza)	5.4	0.075	0.014
Musaka kussa (stuffed squash)	6.0	0.089	0.015
Musaka bazinjan (stuffed eggplant)	5.4	0.077	0.014
Asedah ma'lahm (cooked dough with meat)	7.1	0.069	0.010
Fasuliah (lamb casserole)	5.8	0.070	0.012

Source: Sawaya et al (1986)
[a]: vitamin B-6 to protein ratio was calculated.

Table 1.11
Protein and vitamin B-6 content of six popular cereals and legume-based Saudi dishes

Dish name	Protein (g/100g)	Pyridoxine (B-6) (mg/100g)	B-6:Protein Ratio[a]
Ruz mufalfal (cooked rice)	2.9	0.022	0.008
Medjaddara (cooked rice and lentils)	4.2	0.053	0.013
Sawekah (cooked barley)	2.6	0.078	0.030
Sheariyah (fried vermicelli)	4.6	0.021	0.004
Foul Moudammas (cooked broad beans)	7.5	0.048	0.006
Asedah bil shourbat (cooked dough with soup)	2.7	0.069	0.026

Source: Al-Jebrin et al (1985)
[a]: Vitamin B-6 to protein ratio was calculated.

However, none of the studies reported what basis was used to determine their popularity or frequency of intake. Both studies determined protein content by the AOAC method (1980) and vitamin B-6 content by method of Atkins (1943). Data from these two studies illustrated that vitamin B-6 content, compared to protein, is relatively low, which may indicate that vitamin B-6 status among Saudis is inadequate.

The prevalence of overweight and obesity in Saudi Arabia was reported by Al-Shoshan (1992). This study reported that adult obesity affected women in particular; changes in food habits and lifestyle were a leading cause for this observation. Al-Nuaim et al (1997) found that in a sample of 10,651 adult males and females, the prevalence of those overweight was 31.2% in general, and the prevalence of obesity was 17.8% and 26.6% for males and females, respectively. Another study by El-Hazmi and Warsy (1997) investigated a sample of 14,660 adult Saudi males and females from different regions of the country, and reported that prevalence of persons overweight was 27.2% and 25.2% for males and females, respectively. The prevalence of obesity was 13.1% and 20.3% for males and females, respectively. The study reported that there were several explanations for high prevalence of obesity in Saudi women, including low physical activity and eating snacks during leisure time, especially when the majority of women in the country are not employed. A national evaluation of the nutritional status of Saudis by Al-Nozha et al (1996) examined a sample of 17,892 subjects from all regions of Saudi Arabia and reported that 23-47% and 47-71% of subjects aged 18-30 yr and 30-60 yr, respectively, had a BMI >25

Kg/m^2. This national survey indicated that overweight and obesity is a major health problem in the country and reported that the average energy intake in Saudi population was 3082 kcal/day; dietary fat contribute about 44% of the total average energy intake.

Diabetes mellitus has also become a major health problem in Saudi Arabia (Al-Shoshan, 1992). In a national survey, Al-Nuaim et al (1995) indicated that the prevalence of diabetes (as measured by fasting blood glucose concentration) among adult Saudis was 11.8% in males, and 12.8% in females. In Riyadh city, El-Hazmi et al (1995) surveyed a sample of 2,402 adult males and females and reported the prevalence of diabetes (as measured by fasting blood glucose concentration) was 16% in males and 12.3% in females. These three studies reported that the high prevalence of diabetes among Saudis is related to the rapid socioeconomic changes in the country. Al-Nozha et al (1996) reported that 12.9% of the sample mentioned above had a fasting blood sugar >110 mg/dL. Diabetes mellitus in USA is considered to be one of the most important health concerns, however, a recent National Diabetes Fact Sheet (1998) reported that prevalence of diabetes among the US population is 5.5%. This illustrates the severity of the prevalence of diabetes among Saudis.

Several studies have also reported the prevalence of hypercholesterolaemia among Saudi subjects. Al-Nuaim et al (1995) indicated that the prevalence was 9% and 11% in males and females, respectively. Similar results were reported by Al-Shammari et al (1994), who investigated 1,005 family practice attenders at King Fahad National Guard Hospital in Riyadh, and found 39.3% of patients with a serum cholesterol of 5.2-6.8 mmol/L and 9.5% of patients with serum cholesterol exceeding

6.8 mmol/L. Compared to the reference values of blood cholesterol [5.2-6.2 mmol/L for borderline of high risk and > 6.2 mmol/L for high risk (Grundy, 1999)], the results of this study illustrates the prevalence of hypercholesterolaemia among Saudis. Similar findings were reported by Al-Nozha (1996) who indicated that 28.2% of the sample had a cholesterol level >5.2 mmol/L. A high intake of saturated fat and dietary cholesterol is a leading cause for hypercholesterolaemia among Saudis (Madani et al, 2000). In addition, the content of saturated fat compared to unsaturated fat in common Saudi foods is high; a high amount of saturated fat and dietary cholesterol and a low amount of polyunsaturated fat in popular Saudi foods was reported by Sawaya et al (1986) and Al-Jebrin et al (1985). Analysis of twenty-eight Saudi common foods for their fatty acid content was reported by Al-Khalifa and Al-Othman (1999). The investigators found that polyunsaturated to saturated fat ratio in 17 foods was lower than recommended (0.45).

Anemia, mostly iron deficiency, is a major health problem among Saudi children. Al-Naquib and Sadek (1988) examined hematologic profiles in 208 Saudi children aged 2-60 months, and found that 38% of the children were anemic, 22.6% were borderline, and 39.4% were normal. Similar results were reported by Al-Fawaz (1993) who found that the prevalence of anemia among children in Riyadh city aged 6-24 months was 37%. A recent study by Al-Othaimeen et al (1999) showed that the prevalence of anemia among schoolgirls in Riyadh city, aged 11-18 years, ranged from 48% to 60%. This study, as well as the last two studies, reported that anemia among Saudi children is mainly due to iron deficiency. Several factors contribute to

this deficiency, including low intake of iron and vitamin C, and the high consumption of tea and coca, which contain polyphenols that could inhibit the absorption of non-heme iron in these children (Al-Othaimeen et al, 1999).

Data of nutrient intake and status for Saudis are also very limited. The national survey by Al-Nozha et al (1996) used the 24-hour recall and reported that the average daily consumption of energy, carbohydrates, dietary fiber, total fat and protein for male and female Saudis were 3082 kcal, 300g, 24.4g, 145g and 115g, respectively. These results were the average from both genders of the various age groups. Musaiger (2002) reported that per capita intake of energy and total fat in Saudi Arabia in 1997 was 2783 kcal and 78.6g, respectively.

Al-Shagrawi et al (1995) reported that intake of dietary fiber for female students in King Saud University, Riyadh - Saudi Arabia was 3.4 g/day. Zahran and Zahran (1994) reported non-significant differences in dietary fiber intake between elderly males and females residing in a Riyadh nursing home, 13.2 vs. 13.1 g/day, respectively. Another study by Al-Jassir et al (1996) investigated dietary fiber intake for adult and elderly hospitalized patients in Riyadh region. The result showed that fiber intake of elderly (13.9 g/day) was significantly higher than the intake for younger adults (9.4 g/day).

There are limited studies that reported zinc, riboflavin, vitamin D and calcium status among Saudis. However, none of these studies measured dietary intake. Zinc status was investigated by Al-Ayash (1989) in 57 sickle cell anemia patients and 45 matched disease free control adult Saudis. Results showed no significant differences

between the two groups for plasma levels and urinary zinc excretion as well as serum activity of two zinc dependent enzymes; alkaline phosphatase and lactate dehydrogenase.

Riboflavin status was investigated by El-Hazmi and Warsy (1987) in adult Saudi males (n=289) and females (n=127). This study used glutathione reductase activity coefficient (AC) to determine riboflavin status and defined deficiency as having an AC value $\geq$ 1.3. The investigators found a riboflavin deficiency in 19.7% and 44.9% of male and female participants, respectively. Another study by the same investigators (1989) evaluated riboflavin status using the AC value in adult males and females from three different regions of Saudi Arabia; Al-Hafouf, Jaizan and Riyadh. The results showed that among males, riboflavin was deficient in 32.6%, 17.8% and17.1% of participants from the three regions, respectively. Among females, deficiency was found in 41.5%, 22.3% and 37.9% from the three regions, respectively. These results indicated that riboflavin deficiency was more prevalent among females compared to males. In addition, the results showed a marked variation among the three regions, which could be due to difference in the dietary habits of the subjects in the three regions.

Compared to other nutrients status, vitamin D status has been evaluated in more studies; all of these studies agreed on its deficiency among various groups of the Saudi population. Sedrani et al (1983) investigated serum levels of the circulating form of vitamin D (25-hydroxycholecalciferol [25-(OH) vitamin D]) in adult (26 males and 33 females) and elderly (13 males and 11 females) Saudis. The results showed that 73%

of adult males, 30% of adult females and 83% of the elderly had serum 25-(OH) vitamin D levels below 10 ng/ml (normal range is 10-50 ng/ml). The study also reported that when five elderly hospitalized patients from Riyadh Central Hospital were exposed to sunlight for 5 minutes/day for one week there was a 2.5 fold increase in the level of serum 25-(OH) vitamin D. The researchers concluded that vitamin D deficiency among Saudis is mainly due to avoidance of sunlight exposure. Fonseca et al (1984) investigated a sample of 31 Saudi women and reported that 90% of them had markedly low levels of 25-(OH) vitamin D. Women who live in apartments had significantly lower levels of the vitamin compared to those live in villas or rural areas. This study used a direct question to assess exposure to sunlight and found that levels of 25-(OH) vitamin D were significantly lower in women whose average exposure to sunlight was <30 min/day compared to those with average exposure of > 30 min/day. This study concluded that inadequate exposure to sunlight is a major cause for vitamin D deficiency among Saudi women. Another study by Sedrani (1984a) examined serum levels of 25-(OH) vitamin D in 65 healthy adult Saudi men and found that 35% of subjects had levels below 10 ng/ml. This study indicated a prevalence of vitamin D deficiency among Saudis and recommended food fortification with vitamin D as well as encouragement of exposure to sunlight. Taha et al (1984) reported a deficiency of vitamin D and calcium among babies and pregnant Saudi women. This study measured plasma 25-(OH) vitamin D and calcium concentrations in 100 Saudi mother and their neonates. Plasma calcium concentrations were low in 61% of the mothers and 59% of the neonates. Plasma 25-(OH) vitamin D concentrations were low in 59%

of the mothers and 70% of the neonates. This study also found that 20 babies and 16 mothers had hypocalcemia in the presence of normal levels of 25-(OH) vitamin D, which indicated that vitamin D had a crucial, but not exclusive, role in calcium status of pregnant Saudi women. Sedrani (1984b) indicated a prevalence of vitamin D deficiency and a normal calcium status among adult Saudis. This study measured serum concentration of 25-(OH) vitamin D and calcium in 104 healthy Saudi males aged between 18-23 years. Results showed that 35% of participants had serum 25-(OH) vitamin D concentration below 10 ng/ml. All of participants had normal serum calcium concentration. Abdullah et al (2002) investigated causes of rickets in 34 (20 females and 14 males) adolescent patients by measuring plasma concentrations of 25-(OH) vitamin D and calcium. Results showed that the commonest cause of rickets was vitamin D deficiency (58.8%) followed by hypocalcemia (11.8%).

The above-mentioned studies agreed on the prevalence of vitamin D deficiency among Saudis and attributed this deficiency to inadequate exposure to sunlight; however, dietary intake of vitamin D needs to be investigated.

Smoking habits in Saudi Arabia

Smoking is considered prevalent among Saudis; however, the limited available studies agreed that smoking is an important health problem in Saudi Arabia and recommended the need for tobacco control in the country. A study by Jarallah et al (1999) investigated cigarette smoking in a sample of 8310 subjects from both sexes aged ≥15 years from different regions of Saudi Arabia. Results showed that 21.1% and

0.9% of males and females, respectively were cigarettes smokers. Most of the smokers (78%) were young to middle-aged (21-50 years old). Results of this study illustrated that prevalence of cigarette smoking was higher among uneducated subjects and married people. This study used a direct question to determine smoking status. Since smoking is not socially accepted in the Saudi community, the percentage of smokers reported in this study is expected to be underestimated. Another study by Siddiqui et al (2001) investigated the prevalence of cigarette smoking in a sample of 634 male Saudis aged > 12 years old. The results showed that 34.4%, 16.4% and 49.2% of the sample were current smokers, ex-smokers and non-smokers, respectively. The national survey by Al-Nozha et al (1996) reported that prevalence of smoking among adult male and female Saudis aged >15 years old was 8.4%. Among smokers, 78.2% were cigarettes smokers and 20.4% were water pipe smokers. The small percentage of smokers reported in the national survey compared to the other two studies was due to including female subjects in the sample. Since smoking is rare among Saudi females, results of the national survey underestimated percentage of smoking among Saudi males.

Water pipe is a common type of smoking in Saudi Arabia as well as the Middle East countries. This type of smoking used tobacco leaves that are mixed with fermented fruits, honey or mint to add flavors. The tobacco is covered with aluminum paper, placed in a bowel and toped with a piece of burning charcoal that keeps the tobacco burning. Smoker draw air from the burning tobacco; the air passes a small container of water and then enters a rubber tube and finally delivered to mouth. This

smoking system lacks filters that are usually used with cigarettes. However, water pipe smokers believe that water acts as filter. There is a misconception among water pipe smokers that this type of smoking is a safe alternative to cigarettes smoking, yet there is no scientific evidence to support these assumptions. In the contrary, the limited available studies illustrated that health hazards associated with water pipe smoking were similar to those associated with cigarettes smoking.

Carboxyhemoglobin concentration in blood is used as an indicator of the extent of which the gaseous phase of tobacco smoke reaches the lungs. The carboxyhemoglobin is also used as an indicator of exposure to tobacco smoke. This indicator was measured among adult male Saudis who were non-smokers (n=38), cigarettes smokers (n=32) and water pipe smokers (n=26) (Zahran et al, 1982). The results showed significantly higher blood concentrations of carboxyhemoglobin among water pipe smokers compared to cigarettes smokers as well as among cigarettes smokers compared to non-smokers. Zahran et al (1985) reported similar results. This study examined blood concentration of carboxyhemoglobin in 1832 healthy Saudi males aged between 16-73 years. These subjects were non-smokers (n=256), cigarettes smokers (n=601) and water pipe smokers (n=975). The results showed that blood concentrations of carboxyhemoglobin were significantly higher among water pipe smokers compared to cigarettes smokers as well as among cigarettes smokers compared to non-smokers. This study also reported a significant linear relation between number of cigarettes as well as number of practicing water pipe smoking/ day and blood concentrations of carboxyhemaglobin. Results of the last two studies

suggested that water pipe smokers probably absorb more carbon monoxide than cigarettes smokers since more tobacco smoke is delivered to lungs. Shafagoj et al (2002) measured levels of nicotine and cotinine in saliva, plasma and urine of healthy male water pipe smokers. Smokers were asked to avoid smoking for at least 48 hours prior to first measurement (baseline), and then smoke for 45 minutes before second measurement. The results of the second measurements showed that levels of nicotine and cotinine in saliva, plasma and urine were significantly elevated to high values compared to baseline measurements.

METHODS

Subjects

Fifty-four Saudi adult males were recruited from the Riyadh region of Saudi Arabia. Thirty-two of these individuals were urban subjects living in Riyadh City, and twenty-two were rural subjects, living in towns or villages near the city. The recruitment was done by personal contact, presentations in governmental work centers and mosques as well as by advertisement; a translation of the advertisement poster is given (Appendix C, Figure C.1). All volunteers received a questionnaire to determine their eligibility for participation in the study. A translation of the questionnaire is given (Appendix C, Figure C.2). The questionnaire asked for age, body weight, height, exercise habits, health status, and smoking habits, and the use of dietary supplements. Users of dietary supplements or drugs that are known to affect vitamin B-6 metabolism, absorption or urinary excretion (Bhagavan, 1985) were excluded. Only healthy adult males were eligible to participate. Females were excluded from the study because Saudi tradition segregates the two genders. The study protocol was explained to the subjects and informed consent was obtained. The informed consent form is given (Appendix C, Figure C.6). All study procedures with human subjects were approved by Oregon State University institutional review board for the protection of human subjects.

Experimental Protocol

All subjects received written instructions about keeping three days' food records and urine collection. A translation of the instructions is given (Appendix C, Figure C.3). The instructions were discussed with the subjects individually. Moreover, a one-day trial of keeping food record and collection of a 24-hour urine was performed after discussion of the instructions with each subject. This one-day trial was performed five days prior to the experiment and was performed to ensure that all subjects properly followed the experimental protocol.

All subjects recorded their dietary intake and collected complete 24-hour urine for three consecutive days. For each subject, energy requirement (based on his physical activity level, weight and age) and energy intake (based on his food record intake) were determined by the Food Processor (2001). Subject(s) for whom their difference between energy requirement and energy intake exceeded 15% were excluded since this difference indicates incomplete food record. On the fourth day, subjects were asked to come to the nutrition laboratory in a fasting state. Weight and height were measured and BMI was calculated as weight (Kg) / height (m^2). Blood samples were collected in heparinized tubes (Becton Dickins, Rutherford, NJ) and placed on ice. Blood samples were centrifuged at 1800 x g, 4°C for 20 minutes and plasma samples were separated and stored at -20°C until shipment. Red blood cells were washed three times using ice cold 0.9% saline. After mixing the red cells with

saline, the samples were centrifuged at 1800 x g for 20 minutes at room temperature (Miale, 1977). Red blood cell samples were stored at -20°C until shipment.

Daily 24-hour urine samples were collected in brown opaque three-liter polyethylene bottles containing 15 ml of toluene as a preservative. The total volume of each day was measured and three 20 ml samples were obtained and stored at -20°C until shipment.

The above steps were performed in the nutrition laboratory in the Department of Food Sciences and Nutrition, College of Agriculture at King Saud University in Riyadh, Saudi Arabia. Plasma, red blood cells and urine samples were shipped on dry ice to the Department of Nutrition and Food Management at Oregon State University. Upon arrival, the plasma and red blood cells samples were stored at -70°C and urine samples were stored at -20°C until analyses.

Biochemical Analyses

All samples were analyzed in duplicate, and the average is reported if the difference did not exceed 8%. Hematocrit and hemoglobin were determined using microhematocrit and cyanmethemoglobin methods respectively, as described by Miale (1977).

Plasma vitamin B-6 vitamers and red blood cell PLP were analyzed by high performance liquid chromatography (HPLC) using the method of Sharma and Dakshinamurti (1992). The plasma vitamers measured were PLP, 4-PA, PL and PN.

An excitation wavelength of 330 nm and an emission wavelength of 400 nm were used. Two mobile phases were used; buffer A: 0.033M phosphoric acid/8 mM octanesulfonic acid, and buffer B: 0.033M phosphoric acid/isopropanol (18% V:V), both at pH 2.2. The flow rate was 1.0 ml/min. The column eluate was combined with the post column reagent to enhance the fluorescence of PLP, at a flow rate of 0.03 ml/min of 1.0 g/L sodium bisulfite in 1 M KH_2P04, pH 7.5. The HPLC used was a Shimadzu system, which consisted of a controller (SCL–10A), two pumps (LCL-10AD), a 250 µL injection loop, a fluorometer (RF-10A) and a recorder/integrator (CR501). The column was a C18 ion-pair analytical column (3 µm particle size, 4.6 mm x 100 mm, Rainin Instrument Co., Emeryville, CA). The post column pump was a Kd (Kenneth Dunne) scientific syringe pump model 200. Sample preparation was performed under yellow fluorescent lighting to prevent photodegradation of vitamin B-6. A standard and control samples were analyzed with every six samples. The concentration of each B-6 vitamer was calculated using the 0.05 µmol/ml standard peak height.

Alkaline phosphatase activity was determined by colorimetric assay, as described by Roy (1970). This method uses the substrate sodium thymolphthalein and measures absorbance of the liberated thymolphthalein with a buffer pH of 10.15 at 25° c. Absorbance was measured at wavelength of 590 nm. Control and standard samples were analyzed with every eight samples. Plasma albumin was determined using the method of Slater et al (1975). This method measures albumin concentration by color change due to the binding of the dye, bromocresol green, to

albumin. Absorbance was measured at wavelength of 628 nm. Control and standard samples were analyzed every sixteen samples.

Urinary 4-PA excretion was analyzed by a modified HPLC procedure (Gregory and Kirk, 1979), using a phosphate methanol mobile phase (0.034 M, 2.2 pH phosphate buffer; 1.25% acetonitrile, 5% methanol). Excitation wavelength was 320 nm, and the fluorescent emission was 425 nm. Peak area was recorded by a Hewlett-Packard Integrator (3390A). The column was a C18 reverse phase column (10 μm particle size, 4.6 mm x 250 mm). Standard and control samples were analyzed with every ten samples.

Urea nitrogen was measured by automated method as described by DiGiorgio (1974). The basis of this method is that urea reacts with diacetyl monoxime in an acid solution and the presence of ferric ions as oxidizing agent. This reaction produces a chromogen, which is measured photometrically.

Urinary creatinine was measured by automated method as described by Pino et al (1965). The basis of this method is the photometric measurement of the compound formed from the reaction between creatinine and alkaline picrate.

Dietary Intake Analyses

The three-day food records of subjects were analyzed using the Food Processor, version 7.8 (2001) (ESHA Research, Salem, OR) to determine the average intake of vitamin B-6, protein, soluble dietary fiber, insoluble dietary fiber, riboflavin

and zinc. The average intake of energy and other nutrients were also determined, these nutrients were: carbohydrates, total fat, saturated fat, unsaturated fat, cholesterol, vitamin C, vitamin B-1, vitamin D, vitamin B-3, vitamin B-12, folate, calcium and iron. The percentage of animal food that were sources of vitamin B-6 and protein were calculated. For foods that are not in the Food Processor program, other tables of food composition were used to determine the intake of the previous nutrients. Four tables of food composition were used: Nutritional Value of Foods (Musaiger, 2001), Food Composition Tables for use in the Middle East (Pellett and Shadarevian,1970), Table of Food Composition (Whitney and Rolfes, 1999) and The Composition of Foods (Paul and Southgate, 1978). There were thirteen foods that did not have a value for vitamin B-6 content in either the Food Processor or other food composition tables. Four of these foods were spices (Allspice, Black cumin, Black lemon and Zatter) and three were desserts (Halawa tahinea, Basbosa and Sheariya). The rest were dates, Molokia (leafy green vegetable), Jarish (whole wheat chunks), Falafel (fried legume), camel meat and camel milk. A description of these foods will be discussed in the results chapter. These foods were obtained from local market in Riyadh-Saudi Arabia and were shipped to Corvallis, OR in a plastic zipper bags on dry ice and stored at -20°C until analyzed. Before analysis, Molokia, Jarish, Falafel and camel meat were defrosted and cooked. Molokia was boiled in a tap water (ratio 2:1) for 30 min whereas Jarish was boiled in chicken broth (ratio 1:3) for 70 min; after cooking, the excess water was drained. Falafel was fried in corn oil for 8 min and then placed over absorbent papers to remove excess oil. Camel meat was dry roasted in a fry pan for 8

min at 150° F. Spices that came in a powder form and camel milk that was pasteurized were analyzed as such. The remainder of the samples were ground in a Waring Blender using a Waring pulverizer with capacity of 75g. The blender was run for short periods (maximum of 1 min) to avoid heat buildup. Camel meat was frozen in liquid nitrogen before blending to obtain a smooth powder. Vitamin B-6 content of these foods was measured in duplicate and the average is reported if the difference did not exceed 8%. The method was a microbiological assay using the yeast *Saccharomyces uvarum* (AOAC, 1984). This method is based on yeast growth stimulation by pyridoxine. The yeast *Saccharomyces uvarum* was selected because of its specific response to pyridoxine (Atkin et al, 1943).

Statistical Analysis

The S-plus statistical analysis software package version 6.1 (Insightful Corporation, Seattle, WA) was used to analyze all data. Descriptive statistics, which include maximum, minimum, mean, and standard deviation, were used to describe the data for urban, rural, smoker, and non-smoker subjects.

The Welch modified two sample t-test, which adjusts for non-equal variance was used to compare measures between urban and rural subjects, as well as smoker and non-smoker subjects. Comparison between non-smokers, cigarette smokers and water pipe smokers was performed by one way analysis of variance (ANOVA)- Tukey

Kramer procedure. A simple linear regression model was used to determine correlation between measures. Significance of difference was set at p-value of ≤ 0.05.

Descriptive statistics and comparisons were made for the following measures: age, weight, height, BMI, hematocrit, hemoglobin, plasma vitamin B-6 vitamers concentration, red blood cell PLP concentration, plasma albumin concentration, plasma alkaline phosphatase activity, urinary 4-PA, creatinine and urea nitrogen excretion and nutrients intake.

RESULTS

Subject Characteristics

There were 54 participants in the study; 32 were urban subjects, and 22 were rural subjects. During the data collection, one urban subject and one rural subject did not follow the study protocol and consumed breakfast before the blood draw. A third rural subject used a medication during the 3-day urine and food record collection and was also the only subject whose difference between energy requirement and energy intake exceeded 15%. Statistical analysis of vitamin B-6 indices concentration in plasma and urine showed that their indices were extreme outliers. Although these three subjects were excluded from the analysis of vitamin B-6 status, two of them were included in the analysis of nutrient intake. The 51 participants, 31 urban and 20 rural, were reclassified to smokers (n=24) and non-smokers (n=27). Among smokers, 19 subjects smoked cigarettes whereas 5 subjects were water pipe smokers. Of the 24 smokers, 5 subjects were rural (20.8%) whereas 19 subjects were urban (79.2%).

Questionnaire results for physical activity status showed that among urban subjects, twenty (64.5%) described themselves as sedentary, seven subjects (22.6%) as light active, three subjects (9.7%) as moderately active and one subject (3.2%) as highly active. Among rural subjects, eleven (55%) described themselves as sedentary, eight subjects (40%) as light active, one subject (5%) as moderately active and no one described himself as highly active. Means, maximum and minimum of age, weight,

height and body mass index (BMI) for urban and rural subjects is shown in Table 2.1 and for smokers and non-smokers in Table 2.2. No significant differences were observed between groups for any of these characteristics.

BMI is used as an indicator of weight status. The National Heart, Lung and Blood Institute (1998) classifies BMI in the following categories: <18.5: underweight, 18.5-24.9: normal, 25-29.9: overweight, 30-39.9: obesity and ≥40: extreme obesity. Using these categories, weight status for various groups was as follows; in the urban group, 25.8% (n=8) were overweight, 29% (n=9) were obese and 45.2% (n=14) were normal. In the rural group, 35% (n=7) were overweight, 30% (n=6) were obese and 35% (n=7) were normal. When subjects were reclassified based on smoking status, 20.8% (n=5) of smokers were overweight, 25% (n=6) were obese and 54.2% (n=13) were normal, whereas among non-smokers, 37% (n=10) were overweight, 33.4% (n=9) were obese and 29.6% (n=8) were normal.

Table 2.1
Mean (± SD), max and min of age, height, weight and BMI for urban and rural subjects.

	Urban (n=31)			Rural (n=20)		
	Mean±SD	**Max**	**Min**	**Mean±SD**	**Max**	**Min**
Age (yrs)[a]	33 ± 7	48	20	31 ± 6	46	23
Height (cm)	172 ± 6	187	164	171 ± 7	185	160
Weight (kg)	80.0 ± 17.8	124.0	51.6	83.1 ± 19.5	122.0	55.0
BMI (kg/m²)	27.1 ± 5.5	41.4	19	28.2 ± 6.0	38.4	20.5

[a] : age was reported in Arabic calendar, year = 354 days.

Table 2.2
Mean (± SD), max and min of age, height, weight and BMI for smokers and non-smokers

	Smokers (n=24)			Non-smokers (n=27)		
	Mean± SD	Max	Min	Mean± SD	Max	Min
Age (yrs)[a]	33 ± 6	48	24	32 ± 7	46	20
Height (cm)	172 ± 7	187	160	171 ± 6	185	163
Weight (kg)	80.0 ± 19.2	124.0	51.6	83.2 ± 17.7	122.0	58.0
BMI (kg/m²)	26.5 ± 5.8	41.4	19.0	28.4 ± 5.4	38.4	20.5

[a]: age was reported in Arabic calendar, year = 354 days.

Vitamin B-6 content in some Saudi foods

Food records obtained from all subjects revealed 13 foods and spices for which there was no published value for vitamin B-6 content. The foods and their content of vitamin B-6 are shown in Table 2.3. Allspice is very popular and frequently used in Saudi foods. All of the subjects reported it in their food records and consumed an average of 0.8 g/day. Black cumin, black lemon and zatter (thyme with sesame seeds) were spices that were consumed less frequently compared to Allspice. The percentage of urban subjects who reported these three spices in their food records were 41.9%, 51.6% and 19.4%, respectively, whereas the percentage of rural subjects were 55%, 30% and 10%, respectively. Halawa tahinea (sesame with syrup and pistachio), Basbosa (cooked wheat semolina with syrup, yogurt and coconut) and Sheariya (fried hair pasta with syrup and cardamom) are common desserts. The syrup used in all of them was sugar syrup, which is made of white cane sugar and water in a ratio of 2:1.

The percentage of urban subjects who reported these three desserts in their food records were 25.8%, 9.7% and 6.4%, respectively, whereas percentages of the rural subjects consuming these foods were 20%, 0% and 5%, respectively. Dates are the most popular fruit among Saudis; all participants reported consumption of dates. Although the value of vitamin B-6 content in dates has been published (the Food Processor, 2001), the popular date species consumed in the Riyadh region was analyzed to determine if there was a possible variation among date species. The vitamin B-6 content of dates as reported in the Food Processor (2001) is 0.19 mg/100g and in this study (Table 2.3) was 0.23 mg/100g; the vitamin B-6 content of dates found in this study was used in determining total intake of vitamin B-6. Molokia (jew's mallow) is a leafy green vegetable that is usually cooked by boiling with other ingredients. The percentage of the subjects who reported consuming Molokia was 9.7% and 5% from the urban and rural areas, respectively. Jarish is a cereal based dish that is made from chunks of whole wheat cooked by boiling in a chicken broth and cultured buttermilk in a ratio of 3:1. Consumption of Jarish was reported by 45.2% and 55% of the urban and rural subjects, respectively. Falafel is a popular fried legume food among Saudis that is usually consumed with bread as a sandwich; it is made of mashed chickpeas (also can be made from broad beans) with various leafy vegetables and spices. It was reported by 58.1% and 50% of the urban and rural subjects, respectively. Camel meat is the second most popular red meat consumed by Saudis after mutton; 64.5% and 80% of the urban and rural subjects, respectively, reported

consuming camel meat. Camel milk was rarely consumed; it was reported in the food record of only one rural subject.

Table 2.3
Vitamin B-6 content of some common Saudi foods and spices

Food Name	Vitamin B-6 (mg/100g)
Allspice	0.52
Black cumin	1.01
Black lemon	0.21
Zatter	0.73
Halawa tahinea	0.39
Basbosa	0.05
Sheariya	0.04
Dates	0.23
Molokia	0.07
Jarish	0.31
Falafel	0.15
Camel meat	0.45
Camel milk	0.05

Dietary Intake

The food records indicated that most participants from urban and rural groups consumed three meals /day; lunch was the main meal for all participants. Among the urban group, the percentage of subjects who reported skipping meals was 16.1% for breakfast and 25.8% for dinner. In the rural group, these percentages were 15% for breakfast and 25% for dinner. No subject from either group reported skipping lunch. The percentage of subjects who reported eating between meals was 71% and 70% for the urban and rural groups, respectively. The most frequently consumed foods that

were reported by >90% of each urban and rural subject were mutton, rice, wheat bread, dates and tomatoes. Other foods that were reported by >70% of the subjects were chicken, camel meat, cucumbers, potatoes, broad beans, watermelon, white cheese and yogurt. The predominant drink consumed with meals was cultured buttermilk; between meals, sweet black tea was the favorite drink. Soda and coffee were consumed to a lesser extent. Arabic coffee – lightly roasted coffee beans with cardamom in a ratio of 2:1– was consumed more frequently, compared to American style coffee. The vitamin B-6 content of cardamom is 0.23 mg/100g (Leonard et al, 2001). Since Arabic coffee is served in a small cup that contains approximately 30 ml and a coffee pot that contains 30g cardamom can serve approximately 34 cups, the cup of Arabic coffee contains approximately 0.002 mg of vitamin B-6. Thus, Arabic coffee is not considered as a significant source of vitamin B-6. Since food records were collected during the summer season, which is the harvest season for several foods such as watermelon, dates and cantaloupe, intake of these foods may be higher during summer season compared to other seasons. Thus, a seasonal variation in intake may be expected.

Means, maximum and minimum intake for urban and rural subjects of macronutrients and selected micronutrients are shown in Table 2.4. No significant differences were observed between the two groups in the mean intake of these nutrients except for vitamin D ($p \leq 0.05$) and calcium ($p \leq 0.02$), for which the mean intakes were significantly higher in the urban group compared to that of the rural group.

Table 2.4
Mean (± SD), max and min intake of macronutrients and selected micronutrients of urban and rural subjects

Nutrient	Urban (n=32) Mean[a] ±SD	Max	Min	Rural (n=21) Mean[a] ±SD	Max	Min
Energy (kcal)	2460 ± 404[1]	3141	1819	2504 ± 415[1]	3356	1935
Carbohydrates (g)	329 ± 75[1]	496	217	339 ± 71[1]	567	249
Total fat (g)	85 ± 27[1]	143	14	84 ± 23[1]	126	37
Saturated fat (g)	28 ± 13[1]	58	4	27 ± 10[1]	48	12
Unsaturated fat (g)	46 ± 15[1]	78	9	47 ± 13[1]	64	20
Protein (g)	101± 23	141	49	104± 29	169	69
Cholesterol (mg)	361 ± 157[1]	662	18	403 ± 149[1]	718	176
Vitamin B-6 (mg)	2.18± 0.62	3.90	1.42	2.15± 0.65	3.73	1.31
Vitamin C (mg)	147 ± 82[1]	465	38	130 ± 89[1]	331	9
Vitamin B-1 (mg)	1.6 ± 0.4[1]	2.5	0.8	1.6 ± 0.5[1]	2.8	0.7
Vitamin D (IU)	58 ± 61[1]	300	15	35 ± 19[2]	98	20
Vitamin B-3 (mg)	29.7 ± 9.8[1]	55.1	16.1	33.9 ± 10.4[1]	57.7	14.4
Vitamin B-12(µg)	7.8 ± 10.5[1]	47.9	0.8	4.1 ± 2.8[1]	11.9	1.5
Folate (µg)	222 ± 118[1]	686	79	201 ± 67[1]	375	71
Calcium (mg)	974 ± 555[1]	3473	412	714 ± 230[2]	1224	365
Iron (mg)	13.4 ± 6.2[1]	43.8	8.5	12.1 ± 3.9[1]	27.6	8.0

[a] : Means are the average intake of three consecutive days
Different numbers in a given row denote a significant difference, $p \leq 0.05$

The acceptable macronutrient distribution ranges (AMDRs) for adult individuals that have been established in the new DRI (2002) for carbohydrates and fat were 45-65 and 20-35 percentage of energy for adults, respectively. Mean intake of carbohydrates and fat as percentage of energy for the urban group were 53% and 31%, respectively. Nearly identical percentages for carbohydrates and fat were seen for the rural group (54% and 30%, respectively).

Recommendations for prevention of coronary heart disease (CHD) reported restriction of dietary cholesterol intake to less than 300 mg/day and saturated fat to less than 10% of total energy (WHO, 1990; Hayes, 1997). The percentage of the mean intake of total energy as saturated fat for the urban and rural groups was 10.1% and 9.8%, respectively. Based on the recommended values of the DRI, mean intake for the urban and rural groups was adequate for vitamin C, vitamin B-1, vitamin B-3, vitamin B-12 and iron. On the other hand, mean intake in both groups was lower than the DRI recommendation for vitamin D, folate and calcium. The DRI values of these nutrients for adult male and percentage of urban and rural subjects whom their intake was below DRI value is shown in Table 2.5.

Table 2.5
DRI and percentage of urban and rural subjects with intakes below DRI value

Nutrient	DRI	Urban (n=32)	Rural (n=21)
Vitamin B-1	1.2 mg	19%	19%
Vitamin D	200 IU	97%	100%
Vitamin B-3	16 mg	0%	5%
Vitamin B-12	2.4 µg	19%	24%
Folate	400 µg	94%	100%
Calcium	1000 mg	72%	95%
Iron	8 mg	0%	0%
Vitamin C	90 mg	25%	38%

The mean intakes of vitamin B-6, protein and other nutrients that can affect vitamin B-6 status are shown in Table 2.6. The mean intake of vitamin B-6 was adequate for both urban and rural subjects, based on the recommended value of DRI (1.3 mg/day for adult male). None of the participating subjects from either group had

an average intake of vitamin B-6 below 1.3 mg/day. Mean intake of the vitamin was slightly higher in urban subjects compared to rural subjects, but this difference was not statistically significant. The AMDR for protein intake for adults as reported by the DRI (2002) is 10-35% of energy. The mean intake of protein as a percentage of energy for the urban and rural groups was within the normal range (16.6 and 16.7%, respectively). However, due to the high intake of energy, the mean intake of dietary protein was high in both groups with no significant difference between them. This high intake of protein resulted in a lower B-6 to protein ratio of less than 0.020 in fourteen urban subjects, and seven rural subjects, (45% and 35%, respectively). However, the means of the ratios indicated adequate status of vitamin B-6 in both groups according to the suggested value (>0.020) reported by Leklem (2001), with no significant difference between the two groups. The sources of vitamin B-6 and protein as a percentage from animal foods was more than that from plant foods in both groups, and it was significantly higher in the rural group compared to the urban group for both vitamin B-6 and protein, ($p \leq 0.01$ and ≤ 0.02, respectively). The mean intakes of other nutrients that may affect vitamin B-6 status (riboflavin, zinc, soluble and insoluble dietary fiber) are also listed in Table 2.6. Based on the values of the new DRI for adult male, 1.3 mg/day and 11 mg/day for riboflavin and zinc, respectively, mean intakes of riboflavin for both urban and rural groups were adequate whereas means of zinc intake for both groups were slightly less than the recommendation. None of the urban subjects had riboflavin intake below the recommendation of the DRI, whereas among rural subjects, two subjects (10%) had intakes below the DRI. The percentage of urban

and rural subjects who had zinc intake below the recommendation of the DRI was 71% and 75%, respectively. Compared to rural subjects, urban subjects had a significantly higher mean intake of riboflavin, ($p \leq 0.01$), and a higher intake of zinc, but the difference was not statistically significant. Urban subjects had a higher intake of dietary fiber (both soluble and insoluble) than did rural subjects, but the difference was not significant. Recommendations for total dietary fiber intake ranged from 20-40 g/day (Groff et al. 1995); recent DRI (2002) recommended an intake of 38g/day for adult male. Based on these recommendations, neither group had an adequate intake of total dietary fiber.

Mean intake of vitamin B-6, protein and other nutrients that can affect vitamin B-6 status for cigarette smokers, water pipe smokers and non-smokers is shown in Table 2.7. No significant differences between the three groups were observed except for vitamin B-6 intake. Water pipe smokers compared to the other two groups had significantly higher mean intake of vitamin B-6 ($p \leq 0.04$) and lower mean intake of protein, but differences were not significant. These differences caused a significantly higher B-6 to protein ratio in the water pipe smoking group compared to the other two groups ($p \leq 0.01$). Moreover, the contribution of animal foods to vitamin B-6 and dietary protein was significantly lower in the water pipe smoking group compared to the other two groups ($p \leq 0.01$ and $p \leq 0.05$, respectively). Comparison between cigarette smokers and non-smokers groups showed no significant differences in mean intake of vitamin B-6, protein and B-6 to protein ratio. The mean intake of vitamin B-6 and B-6 to protein ratio were adequate for the three groups. No subject from the three groups

Table 2.6
Mean[a] (± SD) intake of vitamin B-6, protein and other nutrients for urban and rural subjects

Nutrient and sources	Urban (n=31)	Rural (n=20)
Vitamin B-6 (mg)	2.18 ± 0.62[1]	2.15 ± 0.65[1]
Protein (g)	101 ± 23[1]	104 ± 29[1]
B-6: protein ratio (mg/g)	0.022 ± 0.008[1]	0.021 ± 0.004[1]
% of B-6 from animal source	50.1 ± 12.0[1]	57.6 ± 9.0[2]
% of dietary protein from animal source	63.0 ± 10.1[1]	69.5 ± 8.5[2]
Riboflavin (mg)	2.34 ± 0.92[1]	1.81 ± 0.43[2]
Zinc (mg)	10.4 ± 3.3[1]	9.8 ± 2.0[1]
Soluble dietary fiber (g)	3.9 ± 1.5[1]	4.4 ± 2.0[1]
Insoluble dietary fiber (g)	9.3 ± 4.4[1]	10.6 ± 5.6[1]

[a]: Means are the average intake of three consecutive days
Different numbers in a given row denote a significant difference, $p \leq 0.05$

had an average B-6 intake below 1.3 mg/d, however, the B-6 to protein ratio was below 0.020 for 11 cigarette smokers and 10 nonsmokers, (45.8% and 37.0%, respectively) due to a higher protein intake. Water pipe and cigarette smokers had a non-significant higher mean intake of riboflavin and zinc compared to non-smokers. Based on the DRI values, mean intake of riboflavin was adequate in all groups, whereas the mean intake of zinc was slightly less than the recommendation in all groups. Also, the mean intake of total dietary fiber for all groups was less than the recommendation of the DRI (2002).

Table 2.7
Mean[a] (±SD) intake of vitamin B-6, protein and other nutrients for cigarette and water pipe smokers and non-smokers

Nutrient and source	Non-smokers (n=27)	Cigarette smokers (n=19)	Water pipe smokers (n=5)
Vitamin B-6 (mg)	2.18± 0.69[1]	2.08± 0.64[1]	2.51± 0.73[2]
Protein (g)	105± 30[1]	101± 19[1]	93± 36[1]
B-6: protein ratio	0.021± 0.004[1]	0.021± 0.005[1]	0.030± 0.016[2]
% of B-6 from animal source	55.7± 8.9[1]	52.8± 10.1[1]	39.6± 19.4[2]
% of protein from animal animal source	67.8± 8.4[1]	64.9± 8.0[1]	56.2± 18.5[2]
Riboflavin (mg)	1.96± 0.52[1]	2.38±1.10[1]	2.12± 0.71[1]
Zinc (mg)	9.6± 2.2[1]	10.9± 3.0[1]	10.3± 5.8[1]
Soluble dietary fiber (g)	4.4± 1.7[1]	3.8± 1.6[1]	4.0± 1.9[1]
Insoluble dietary fiber (g)	10.7± 4.8[1]	8.0± 3.2[1]	12.1± 8.8[1]

[a]: Means are the average intake of three consecutive days
Different numbers in a given row denote a significant difference, $p \leq 0.05$

Hematocrit and hemoglobin

Data for hematocrit and hemoglobin for urban and rural subjects is shown in Table 2.8, and for smokers and non-smokers in Table 2.9. Acceptable values for hematocrit and hemoglobin for adult males are 40-54% and 140-180 g/L, respectively; a deficiency of iron is characterized by values of ≤40% and ≤135 g/L, respectively (Lee and Nieman, 1996). Results for these two measures for urban and rural subjects were in the normal range, with no significant difference between their means. For smokers and non-smokers, hematocrit and hemoglobin values were also within the normal range but the means of both hematocrit and hemoglobin were significantly higher in smokers compared to non-smoker, ($p \leq 0.05$ and ≤ 0.03, respectively).

Table 2.8
Mean (± SD) of hematocrit and hemoglobin concentration for urban and rural subjects

	Urban (n=31)	Rural (n=20)
Hematocrit (%)	48 ± 3	50 ± 3
Hemoglobin (g/L)	162 ± 9	165 ± 12

Table 2.9
Mean (± SD) of hematocrit and hemoglobin concentration for smokers and non-smokers

	Smokers (n=24)	Non-smokers (n=27)
Hematocrit (%)	50 ± 3[1]	48 ± 3[2]
Hemoglobin (g/L)	167 ± 11[1]	160 ± 9[2]

Different numbers in a given row denote a significant difference, $p \leq 0.05$

B-6 vitamers, urinary 4-PA and creatinine and alkaline phosphatase

The mean concentration of plasma B-6 vitamers, red blood cell (RBC) PLP, urinary 4-PA and creatinine excretion and alkaline phosphatase activity for urban and rural subjects are listed in Table 2.10. The mean concentration of plasma PLP and mean urinary 4-PA excretion of both groups indicated an adequate status according to the suggested values for adequate status by Leklem (2001). There were no significant differences between the two groups for any of the B-6 vitamers.

Table 2.10
Mean (±SD) concentration of plasma B-6 vitamers, red blood cell (RBC) PLP, urinary 4-PA and creatinine excretion and alkaline phosphatase activity for urban and rural subjects.

	Urban (n=31)	Rural (n=20)
Plasma PLP (nmol/L)	39.3 ± 18.0	40.5 ± 14.6
Plasma PL (nmol/L)	14.0 ± 6.3	12.5 ± 4.4
Plasma PN (nmol/L)	8.8 ± 3.4	9.5 ± 2.8
Plasma 4-PA (nmol/L)	13.5 ± 4.7	12.5 ± 5.6
RBC PLP (nmol/L)	29.9 ± 8.1	26.7 ± 4.7
Urinary 4-PA (μmol/day)[a]	4.6 ± 2.3	4.4 ± 2.3
Urinary creatinine (g/day)[a]	1.5 ± 0.3	1.3 ± 0.3
Urinary 4-PA/creatinine ratio (μmol/g)	3.1 ± 1.8	3.3 ± 1.7
Plasma alkaline phosphatase (U/L)	25.9± 8.5	26.3± 6.3

a: average of three consecutive days.

Although the mean concentration of plasma PLP indicated an adequate status for both groups, nine subjects (29%) of urban group had a concentration below 30 nmol/L. Three of which also had a urinary 4-PA concentration below 3.0 μmol/day; an additional four subjects also had a concentration below 3.0 μmol/day, a total of seven subjects (22.6%). In the rural group, five (25%) and four (20%) subjects had concentrations of plasma PLP below 30 nmol/L and urinary 4-PA below 3.0 μmol/day, respectively. Vitamin B-6 intake was significantly correlated with both plasma PLP concentration and urinary 4-PA excretion, ($r = 0.33$, $p \leq 0.017$), ($r = 0.33$, $p \leq 0.019$), respectively. Plasma PLP was significantly correlated with BMI ($r = 0.36$, $p \leq 0.01$). The normal range for urinary creatinine excretion in adult male is 1.3-1.9 g/day (Lee and Nieman, 1996). The results in Table 2.10 showed that mean values for

both groups were at the lower normal range with no significant difference between them. Urinary creatinine excretion was significantly correlated with body weight ($r = 0.54$, $p \leq 0.002$). The mean plasma alkaline phosphatase activity for the urban and rural groups was within the normal range (13-39 U/L) (Lee and Nieman, 1996) with no significant difference between the two groups. None of the participants from either group was below or above the normal value of alkaline phosphatase activity. Plasma alkaline phosphatase activity was negatively correlated with plasma PLP, but this correlation was not significant.

The means of plasma B-6 vitamers concentration, RBC PLP concentration, and urinary excretion of 4-PA and creatinine for cigarette and water pipe smokers and non-smokers are shown in Table 2.11. The water pipe smoking group compared to the other two groups had a higher intake of vitamin B-6, which resulted in a significant higher mean concentration of plasma PLP ($p \leq 0.04$), PL ($p \leq 0.01$), red blood cell PLP ($p \leq 0.04$) and urinary 4-PA excretion ($p \leq 0.04$). No significant differences were observed between the three groups for the rest of B-6 vitamers. The cigarette smokers compared to the non-smokers had significantly lower plasma PLP concentration; no significant differences were observed between these two groups for the rest of B-6 vitamers. The mean plasma PLP concentration and urinary 4-PA excretion for the three groups indicated an adequate status of vitamin B-6. However, among the non-smokers, five subjects (18.5%) had plasma PLP concentration lower than 30 nmol/L. One of which, in addition to four subjects (total of five, 18.5%) had a urinary 4-PA excretion lower than 3.0 μmol/day. Among cigarette smokers, nine subjects (47.4%)

had a plasma PLP concentration lower than 30 nmol/L. Two of which, in addition to three subjects (total of five, 26.3%) had a urinary 4-PA excretion lower than 3.0 μmol/day. None of the water pipe smokers had a plasma PLP concentration or urinary 4-PA excretion below 30 nmol/L and 3.0 μmol/day, respectively.

Table 2.11
Mean (± SD) concentration of plasma B-6 vitamers, RBC PLP, urinary 4-PA and creatinine excretion and alkaline phosphatase activity for cigarette and water pipe smokers and non-smokers.

	Non-smokers (n= 27)	Cigarette smokers (n=19)	Water pipe smokers (n=5)
Plasma PLP (nmol/L)	40.0± 12.9[1]	30.9± 12.5[2]	54.9± 23.1[3]
Plasma PL (nmol/L)	12.2± 3.7[1]	13.4± 5.7[1]	21.5± 10.0[2]
Plasma PN (nmol/L)	9.4± 3.4[1]	9.0± 2.9[1]	7.8± 2.3[1]
Plasma 4-PA (nmol/L)	12.9± 4.5[1]	12.1± 4.4[1]	17.7± 8.2[1]
RBC PLP (nmol/L)	28.8± 6.9[1]	27.2± 6.9[1]	33.7± 8.5[2]
Urinary 4-PA (μmol/day)	4.3± 1.2[1]	4.2± 2.4[1]	6.9± 4.7[2]
Urinary creatinine (g/day)	1.4±0.3[1]	1.5± 0.4[1]	1.4± 0.2[1]
Urinary 4-PA: creatinine ratio (μmol/g)	3.0± 0.7[1]	2.9± 1.8[1]	5.0± 3.9[2]
Plasma alkaline phosphatase (U/L)	25.7± 7.7[1]	27.7± 7.7[1]	21.6± 6.5[1]

a: average of three consecutive days.
Different numbers in a given row denote a significant difference, $p \leq 0.05$

Mean creatinine excretion for the three groups was at the lower normal range with no significant differences. However, the higher mean of urinary 4-PA excretion in the water pipe smoking group compared to the other two groups resulted in a significantly higher 4-PA to creatinine ratio. The mean plasma alkaline phosphatase activity for the three groups was within the normal range with no significant difference. None of the

subjects of the three groups had plasma alkaline phosphatase activity below or above the normal range.

Albumin and urinary urea nitrogen

The mean albumin concentration for the urban and rural groups (Table 2.12) was slightly above the normal range (35-50 g/L) (Lee and Nieman, 1996) with no significant difference between the two groups. None of the participants from either group was below the normal value of this measure. The same trend was observed for smokers and non-smokers (Table 2.13). A positive correlation between plasma albumin concentration and plasma PLP concentration was observed, but the correlation was not significant. Means of urinary urea nitrogen excretion for urban and rural subjects (Table 2.12) and for smokers and non-smokers (Table 2.13) were within the normal values (2.2g/day and 14.7g/day for low and high protein diet, respectively) (Pi-Sunyer and Woo, 1984). There were no significant differences between any of the groups. Urinary urea nitrogen was significantly correlated with dietary protein intake, ($r = 0.30$, $p \leq 0.03$). Table 2.14 summarizes the significant correlation coefficients that were reported in this chapter.

Table 2.12
Mean (± SD) of plasma albumin concentration and urinary urea nitrogen excretion for urban and rural subjects.

	Urban (n=31)	Rural (n=20)
Plasma albumin (g/L)	52 ± 3	53 ± 2
Urinary urea N (g/day)[a]	8.6 ± 2.4	7.9 ± 2.4

a: average of three consecutive days.

Table 2.13
Mean (± SD) of plasma albumin concentration and urinary urea nitrogen excretion for smokers and non-smokers.

	Smokers (n=24)	Non-smokers (n=27)
Plasma albumin (g/L)	53 ± 2	52 ± 3
Urinary urea N (g/day)[a]	8.3 ± 2.7	8.4 ± 2.1

a: average of three consecutive days.

Table 2.14
Summary of the significant correlation coefficients

	Vitamin B-6 intake	BMI	Body weight	Protein intake
Plasma PLP	0.33	0.36		
Urinary 4-PA	0.33			
Urinary creatinine			0.54	
Urinary urea N				0.30

DISCUSSION

The major finding of this study was the adequate status of vitamin B-6 of urban and rural subjects as measured by the means of vitamin B-6 intake, B-6 to protein ratio, plasma PLP concentration and urinary 4-PA excretion. In addition, the mean plasma PLP concentration of cigarette smokers was found to be lower than that of non-smokers. Results of this study included other data that can be classified into four categories. The first is demographical data that include smoking habits, exercise habits, meal skipping and BMI. The second is the vitamin B-6 content of Saudi foods. The third is the dietary intake of macronutrients and selected micronutrients in addition to nutrients that affect vitamin B-6 status. The fourth is biological data that include hematocrit, hemoglobin, plasma albumin and urinary excretion of creatinine and urea nitrogen. These categories, as well as comparison between various groups (urban vs. rural and smokers vs. non-smokers), will be discussed in the order mentioned above followed by discussion of vitamin B-6 status for urban and rural subjects and for smokers and non-smokers.

Demographical Data

Just over 47% of the participants were smokers; however, previous studies of smoking among adult Saudi population (Jarallah et al, 1999; Siddiqui et al, 2001) reported lower percentages (21.1% and 34.4%, respectively) than found in this study.

The difference may be due to sampling procedure; this study recruited the sample from free-living adults whereas the other two studies recruited their samples from primary health care attenders. It is most likely that some of the non-smokers were ex-smokers since they were recruited from health care centers. Another reason for the difference is the sample size; this study investigated 51 subjects whereas the other two studies investigated 8310 and 634 subjects, respectively. Of smokers, the percentage of water pipe smokers reported in this study (20.8%) is in agreement with the percentage reported in the national survey (20.4%) (Al-Nozha et al, 1996). These percentages are important in evaluation prevalence of smoking and the health hazards associated with smoking among Saudis. In Saudi Arabia, there is no policy yet for tobacco control except the recent increase (50%) in tobacco taxes. The high prevalence of smoking among participants of this study indicates that there is an urgent need for public health program as well as governmental policies to reduce the rate of smoking among Saudis.

Physical activity status was not determined in this study; however, the majority of participants described themselves as sedentary or lightly active. Low physical activity levels among adult Saudi males were reported by Al-Sudairy and Howard (1992a) and Hakim et al (2003). Although health benefits associated with exercise are well known, exercise habits are not common among Saudis, which indicates a lack of awareness of the importance of exercise.

The food records showed that most participants from urban and rural groups consumed three meals/day and lunch was the main meal. This is in agreement with

previous findings (Al-Shoshan, 1992; Al-Nozha et al, 1996). The percentages of participants who reported eating between meals and skipping meals in this study are in agreement with results of Al-Sudairy and Howard (1992a and 1992b). An exception to this is the percentage of those skipping lunch; this study found that none of the participants skipped lunch whereas Al-Sudairy and Howard (1992b) reported that 15% of 452 adult Saudi students skipped lunch. This difference may be due to difference in sampling; the previous study investigated dietary habits among technical and vocational students who were more likely to skip lunch compared to employees that were investigated in this study. It is known that skipping meals will reduce the chance of meeting nutritional requirements. However, a greater percentage of both urban and rural participants, including all subjects who skipped breakfast and dinner, reported eating snacks between meals, which may complement the skipped meals. On the other hand, skipping breakfast is more critical, because it is a factor for non-replenishment the lowered morning blood glucose level, which may affect mental concentration (Al-Sudairy and Howard, 1992b). Although small percentage of participants of this study reported skipping breakfast, this result indicates the need for nutritional education.

The BMI data revealed a high prevalence of overweight and obesity in both urban and rural subjects. This is in agreement with several previous studies conducted on Saudi subjects (Al-Nuaim et al, 1997; El-Hazmi and Warsy, 1997; Madani and Khashoggi, 1994; Al-Nuaim et al, 1996). Major factors, which may contribute to this prevalence, are high incomes and affluence among Saudis, which lead to a sedentary lifestyle in addition to the lack of nutritional awareness. However, the several studies

that have evaluated overweight and obesity among Saudis used either BMI or weight for height measures. These two anthropometric measures do not indicate the amount of body fat or the location of body fat that are strongly related to health hazards associated with overweight and obesity. Since obesity is one of the major health problems in Saudi Arabia, measures of body fat such as skin fold measurements and measures of fat location such as waist to hip ratio need to be evaluated for Saudis, especially for overweight and obese subjects.

A comparison between smokers and non-smokers for BMI showed no significant differences. This is not in agreement with Albanes et al (1987) who found that adult US smokers had a lower BMI compared to that of non-smokers. On the other hand, the present study is in agreement with Faruque et al (1995) who found no significant difference in BMI between smokers and non-smokers in adult male Bangladesh subjects. BMI as an indicator of overweight and obesity can be influenced by several factors such as age, calories intake and level of physical activity. The two studies mentioned above reported that these factors influenced their results; however, there is no well established mechanism that illustrates the effect of smoking on body weight.

In this study there was a positive significant correlation between BMI and plasma PLP concentration, which is not in agreement with Ledikwe et al (2003). This latter study reported a negative correlation between both BMI and high waist circumference and plasma PLP concentration in elderly males. The difference may be due to the age of the subjects; this study evaluated young to middle aged adults whereas Ledikwe et al evaluated elderly subjects. It has been established that plasma PLP concentration

declines with age (Vanderjagt and Garry, 1985; Hamfelt and Soderhjelm, 1988); thus the difference between this study and Ledikwe study in the correlation is expected. However, an association between BMI and plasma PLP was found in the two studies.

Vitamin B-6 content in Saudi foods

The vitamin B-6 content of the Saudi foods and spices analyzed (Table 2.3) indicates that these foods are relatively poor sources of the vitamin, except for jarish and camel meat. The vitamin contents of the four spices were relatively low compared to other spices (Leonard et al, 2001). The only exception was black cumin (1.01 mg/100g), which can be considered to be relatively high in vitamin B-6. However, these spices are used in very low amounts. Thus, they can't be considered as a significant source of vitamin B-6 in the diet. The higher content of the vitamin in halawa tahinea compared to the other desserts (basbosa and sheariya) was due to the presence of sesame seeds that contain 0.48 mg B-6/100g (Leonard et al, 2001). Dates, which are one of the favored Saudi foods, were the only food in (Table 2.3) that has a published value of vitamin B-6 content (0.19 mg/100g) (Food Processor, 2001). The content of B-6 in dates (Table 2.3) differs from the published data by 21.1%. This difference may be due to variation in the vitamin content among different species of dates. The vitamin content in molokia compared to other vegetables (Table 1.1) was relatively low. Although the vitamin B-6 content of whole wheat – the basic ingredient of jarish – is relatively low (0.08 mg/100g) (Food Processor, 2001), the high B-6 content of jarish was from chicken broth that was used in cooking. The vitamin B-6

content of falafel (Table 2.3) was 6.7% higher than the content of chickpeas (the basic ingredient of falafel) (0.14 mg/100g) (Food Processor, 2001). This difference came from the various spices and herbs that were mixed with chickpeas to prepare the falafel. Camel meat, which is a major source of meat in Saudi diet, contained a relatively higher amount of B-6 (Table 2.3) compared to other meats (Table 1.1). As for other types of meats, camel meat can be considered as a good source of vitamin B-6. In the contrast, camel milk can be considered as a poor source of vitamin B-6. The content of the vitamin in camel milk (Table2.3) was similar to the content of cow's milk (Table 1.1). Of the foods and spices listed in Table 2.3 mixed spices, black cumin, black lemon, dates, jarish, falafel and camel meat were reported by >50% of urban and rural subjects. This illustrates that these foods were common among Saudis. Of these foods, jarish and camel meat contained relatively high amounts of vitamin B-6. Since jarish is prepared from both animal and plant foods, the glycosylated form of the vitamin needs to be determined for jarish.

Dietary Intake

Results of the food records showed that the most frequently consumed foods were mutton, rice, wheat bread, tomatoes and dates. These foods were also reported by Al-Shoshan (1992) and by Al-Nozha et al (1996) as the foods most frequently consumed by Saudis. This illustrate that these foods are basic components of the Saudi diet.

Food records showed that caloric intake was consistent with the energy requirements of the subjects. The records also showed that variations in caloric intake

between subjects was relatively low. This suggests that these subjects were relatively accurate in the completion of their food records. Since this is the first study that has measured dietary intake for adult Saudi males by three-day food records, comparison with other studies is not possible. Dietary intake for Saudis was reported in a limited number of studies by using the food balance sheet (Madani et al, 2000), which does not measure the actual intake. The only exception to this was the national survey by Al-Nozha et al (1996), which used the 24-hour dietary recall. However, the national survey reported only a group average intake for both genders of all age groups.

The mean calorie intake for both urban and rural groups reported in the present study (Table 2.4) was 11% lower than the per capita estimation reported by Musaiger (2002). The main reason for the difference is that per capita estimation counts wasted food in the consumption data, which could overestimat intake. The mean intake of total fat was lower than the mean intake reported in the national survey (145g/day) by 41.4% and 42.1% for urban and rural groups, respectively. The national survey reported that the mean intake of total fat was 145g, which represented 42% of caloric intake. This high intake of fat raised the caloric intake to 3082 kcal, which is above the caloric intake reported in the present study. This could be due to overestimation of the invisible fat in the diet (such as oil that is used in food preparation). The mean intakes of carbohydrate and protein of both groups reported in this study are consistent with the results of the national survey (mean intake of 300g and 115g for carbohydrate and protein, respectively). The mean intake of carbohydrate as percentage of the caloric intake for both urban (54%) and rural (54%) groups was approximately at the middle

of the AMDRs. The mean intake of total fat as percentage of the caloric intake for both urban (31%) and rural (30%) groups was at the high range of the AMDRs, whereas mean intake of protein for the urban (16%) and rural (17%) groups was at the lower range of the AMDRs. The high percentage of protein from animal source reported in this study is in agreement with the national survey, which reported that 60% of dietary protein was from animal sources. This high percentage is also in agreement with other studies that reported a high consumption of red meats and poultry by Saudis (Al-Shoshan, 1992; Miladi, 1998).

There is no available data for adult Saudis intake of saturated fat, unsaturated fat and dietary cholesterol. However, the high intake of dietary cholesterol as well as the high ratio of saturated fat to unsaturated fat found in this study for both urban and rural subjects are consistent with other studies that reported a high prevalence of hypercholesterolaemia among adult Saudis (Al-Shammari et al, 1994; Al-Nuaim et al, 1995). The findings of a high intake of saturated fat and dietary cholesterol indicate the need for increasing nutritional education and awareness of healthy eating among adult Saudi males.

The mean intake of total dietary fiber for both urban and rural groups was markedly below the recommendation, which is in agreement with the findings of Zahran and Zahran (1994) and Al-Jassir et al (1996). Although participants of this study frequently consumed wheat breads, the majority of these breads were made from white wheat flour, which is low in dietary fiber (Al-Kanhal et al, 1999). The dietary fiber content of Saudi diet in general was reported to be low (Al-Shagrawi, 1998). The

latter study reported that methods of food preparation such as peeling vegetables, use of refined wheat instead of whole wheat and low consumption of rich fiber foods were responsible for the low intake of dietary fiber among Saudis. Since the results of this study as well as the above mentioned studies indicate that the intake of dietary fiber among Saudis is low, an increase in the intake of fiber is recommended. However, dietary fiber inhibits zinc absorption and since the mean intake of zinc found in this study was lower than DRI recommendation, the recommendation of increased dietary fiber should be combined with an increase in zinc intake.

The mean intake of vitamin D for both groups was well below the DRI recommendation. This is consistent with other studies that reported prevalence of vitamin D deficiency among adult Saudi males (Sedrani, 1984a and 1984b; Abdullah et al, 2002). In this study, only one subject – from the urban group – had an intake of vitamin D above the recommendation. This subject reported consumption of imported cereals that was fortified with vitamin D. This illustrates the need for fortification of commonly consumed foods – such as milk and dairy products – with vitamin D.

The mean intake of calcium was slightly below the recommendation of the DRI for the urban group and markedly below the recommendation for the rural group. Most of the participants from both groups had an intake below the recommendation. There are no available studies that have investigated the intake of calcium for adult Saudi males. However, Abdullah et al (2002) reported that hypocalcemia contributed to 11.8% of rickets, whereas Sedrani (1984b) reported a normal calcium status of adult Saudis. Inadequate intake of calcium reported in this study is based on the

recommendation of the DRI, which recently increased the recommendation from 800 mg to 1000 mg. This recommended level might not be achieved by diet. In the United States, food (such as juices) fortification with calcium and encouragements of milk consumption are common. Such action is needed in Saudi Arabia to increase the intake of calcium.

The mean intakes of riboflavin were 80% and 39% above the DRI recommendation for the urban and rural groups, respectively. This is in line with the high intake of meats (considered as good sources of riboflavin) that were reported by subjects of both groups. The mean intake of riboflavin was significantly higher in the urban group compared to that of the rural group. This was due to higher intake of riboflavin from consumption of lamb liver by four urban subjects compared to one rural subject. Lamb liver contains 4.4 mg riboflavin/100g (Paul and Southgate, 1978). The adequate intake of riboflavin found in the present study is not consistent with the results of El-Hazmi and Warsy (1987 and 1989) who reported riboflavin deficiency among adult Saudi males. The latter two studies did not report dietary intake of riboflavin. El-Hazmi and Warsy (1987) suggested that riboflavin deficiency could be due to one or more of three factors: insufficient dietary intake, lactose intolerance and climatic conditions. Result of this study indicates that insufficient dietary intake is not one of the contributing factors for riboflavin deficiency among adult Saudi males.

The mean intake of zinc for urban and rural groups was slightly below the DRI recommendation. However, the mean intake for both groups (Table 2.6) was above the EAR. Thus, zinc deficiency would not be expected among the two groups based on

their mean intake. This was also illustrated by the normal values of alkaline phosphatase activity (a zinc metalloenzyme) (Table 2.10). Our assumption of normal zinc status agreed with the findings of Al-Ayash (1989) who reported normal zinc status for adult Saudis.

None of the participants from either group had an iron intake below the recommended DRI. High prevalence of anemia due to iron deficiency was reported among Saudi children (Al-Naquib and Sadek, 1988; Al-Fawaz, 1993; Al-Othaimeen et al, 1999). However, no study has reported iron deficiency anemia among adult Saudi males. The present study suggests a normal iron status among adult Saudi males based on the adequate iron intake and the normal levels of hematocrit and hemoglobin.

A search of Medline and Agricola from 1971 to the present did not reveal any study that has evaluated the intake or the status for adult Saudi males of the following nutrients: vitamin C, vitamin B-1, vitamin B-3, vitamin B-12 and folate. Results of this study showed that the mean intakes of vitamin C, vitamin B-1, vitamin B-3 and vitamin B-12 were adequate for both urban and rural groups. However, few urban and rural subjects had an intake of these nutrients lower than the recommendation (Table 2.5). In those subjects whose intakes were inadequate, consumption of less of a variety of foods by these subjects may have contributed to the inadequacy of these nutrients intake, which indicates the need for increasing the nutritional education and awareness of healthy eating among adult Saudi males. In contrast to the above-mentioned nutrients, the mean folate intake of urban and rural groups was markedly below the recommended DRI (400μg). Recently, the United States fortified wheat flour with

folate. Fortification of foods such as bread is needed in Saudi Arabia, especially since bread is one of the basic components of the Saudi diet.

Comparison between the urban and rural groups for the mean intake of macronutrients and the selected micronutrients raised an interesting point. The urban group, compared to rural group had a lower mean intake of macronutrients and a higher mean intake of several micronutrients. The exceptions to this was the mean intake of total fat, which was similar in the two groups and the mean intake of vitamin B-3, which was lower in the urban group compared to rural group. This indicates that the rural group, compared to urban group consumed more meat and starchy foods and less fruits and vegetables. This was also illustrated by the higher percentage of rural subjects, compared to urban subjects, who had an intake of micronutrients below the DRI recommendation (Table 2.5). This observation indicates that lack of nutritional awareness is higher among rural subjects compared to urban subjects. However, these differences in the mean intake between the two groups were not statistically significant except for vitamin D, calcium and riboflavin.

Biological Data

The hematocrit and hemoglobin values for all participants were in the normal range. This is in agreement with the study of Scott (1982) who reported that hematological values for healthy adult Saudi males were normal. As reported earlier, anemia (mainly iron deficiency) is a major health problem among Saudi children (Al-

Naquib and Sadek, 1988; Al-Fawaz, 1993; Al-Othaimeen et al, 1999) but no study has reported a prevalence of anemia among adult Saudi males. However, hematocrit and hemoglobin values are indicators of late stage of anemia because both indicators become abnormal only in the late stages of iron deficiency (Lee and Nieman, 1996).

As expected, smokers, as compared to non-smokers, had a significantly higher mean hemoglobin value, which is in agreement with previous reports (Nordenberg et al, 1990; Lundman et al, 1990). The same trend was observed for the mean hematocrit value, which is also in agreement with previous reports (Giraud et al, 1995; Lee et al, 1987; Vermaak et al, 1990). The elevation of hematocrit by smoking is explained by elevation of carbon monoxide (a major component of cigarette smoke), which reduces oxygen tension in the body; this reduction increases production, maturity and release of erythrocytes from blood forming organs and thus, elevates hematocrit level (Van Liere and Stickney, 1963).

The mean plasma albumin concentrations of urban and rural groups as well as smokers and non-smokers were slightly above the normal ranges. These high values are within the range reported by El-Hazmi et al (1982) for adult Saudi males. The latter study reported that the plasma albumin concentration of adult Saudi males was higher than that for adult Western males.

In the contrast to plasma albumin, and based on the high protein intake, the mean urinary urea nitrogen excretion was lower than expected. The mean urinary urea nitrogen excretion for all groups found in this study was at the lower normal range of adult Saudi males reported by El-Hazmi et al (1982) and by Scott (1982). This is

mainly due to the incomplete 24-hour urine collection of some participants of this study rather than low protein intake. As expected, this study found a significant correlation between urinary urea nitrogen excretion and protein intake. This correlation between urinary urea nitrogen excretion and nitrogen (mainly protein) intake is well established (Lee and Nieman, 1996). Similar to urinary urea nitrogen, the mean creatinine excretion was also at the lower normal range of adult Saudi males reported by El-Hazmi et al (1982), which is again due to the incomplete 24-hour urine collection of some participants of this study. This study found a significant correlation between creatinine excretion and body weight. Creatinine is strongly related to muscle mass (major component of lean body mass) and lean body mass is component of the body weight. Thus, the correlation found in this study is partially in agreement with the results of Forbes and Bruining (1976) who reported a correlation between urinary creatinine and lean body weight.

Vitamin B-6 status of urban and rural subjects

The hypothesis of this study was that vitamin B-6 status for adult Saudi male is inadequate. This hypothesis was based on the low ratio of B-6 to dietary protein in common Saudi dishes that was reported in two studies (Tables 1.10 and 1.11) (Sawaya et al, 1986 and Al-Jebrin et al, 1985). However, the hypothesis was not supported based on measured of vitamin B-6 status. The present study found that the mean intakes of the vitamin B-6 for the urban and rural groups were 68% and 65% above the DRI recommendation, respectively. Vitamin B-6 status was also evaluated by

vitamin B-6 to protein ratio, plasma PLP concentration and urinary 4-PA excretion. The mean of these indices for the urban and rural groups found in this study indicated that vitamin B-6 status is adequate for both groups. The two studies mentioned above that reported low ratio of B-6 to protein in common Saudi foods reported a 90% loss of vitamin B-6 during preparation, which is in conflict with all previous data that reported loss of the vitamin ranged between 15-50% (Leklem, 2001). Moreover, one of the dishes reported in Table 1.11 named Sheariyah, was analyzed in this study (Table 2.3). This study found that the content of the vitamin in the previous dish was 48% above the reported value of Al-Jebrin et al, (1985).

Low riboflavin and zinc status may adversely affect vitamin B-6 status (Sauberlich, 1985; Leklem, 2001). The present study did not investigate riboflavin and zinc status, however, the mean intake of these two nutrients for both the urban and rural groups did not suggest the potential of a deficiency since their mean intakes were above ⅔ of the DRI recommendation. A high intake of dietary fiber may lower the bioavailability of vitamin B-6 as described in the bioavailability section. The mean intake of total dietary fiber for the urban and rural groups was markedly low. This suggests that the affect of dietary fiber on the bioavailability of vitamin B-6 is probably negligible. The mean B-6 to protein ratio indicates an adequate vitamin B-6 status for the urban and rural groups based on the suggested ratio reported by Leklem (1990). However, fourteen urban and seven rural subjects had a ratio below 0.020 due to the high intake of protein. The mean B-6 to protein ratio for these urban subjects was 0.017± 0.002 and their mean plasma PLP concentration was 31.1± 11.7 nmol/L.

The rest of the urban subjects (n= 17), had a mean B-6 to protein ratio of 0.026± 0.009 and a mean plasma PLP concentration of 46.2± 19.6 nmol/L. The seven rural subjects had a mean B-6 to protein ratio of 0.017± 0.002 and a mean plasma PLP concentration of 28.1± 7.1 nmol/L. The rest of the rural subjects (n= 13) had a mean B-6 to protein ratio of 0.023± 0.003 and a mean plasma PLP concentration of 47.2± 13.1. This indicates that the higher protein intake lowered the plasma PLP concentration, which is in agreement with previous studies done in male and female subjects (Miller et al, 1985; Hansen et al, 1996b; Hansen et al, 1997).

The mean plasma PLP concentration for both the urban and rural group was above the suggested value for adequate status reported by Leklem (1990) and correlated significantly with vitamin B-6 intake. This correlation is in agreement with several other reports (Shultz and Leklem, 1981; Lee and Leklem, 1985; Kretsch et al, 1991; Huang et al, 1998). Nine urban and five rural subjects had plasma PLP concentrations below 30 nmol/L. Evaluation these subjects indicated that of the nine urban subjects, seven were cigarette smokers. Two subjects had a relatively low percentage of vitamin B-6 from animal sources (30.6% and 29.6%). Consuming more vitamin B-6 from plant sources could lower the bioavailability of the vitamin, which would lead to a lower plasma PLP concentration. Of the five rural subjects, two were cigarette smokers. Another two subjects had a low B-6 to protein ratio (0.017 and 0.014). The fifth subject had a low percentage of vitamin B-6 from animal source (21.0%), which could lower the bioavailability of the vitamin. The mean plasma PLP concentration found in this study for both the urban and rural groups was marginally lower than

found in other studies in which male subjects were consuming a relatively similar intake of vitamin B-6. Shultz and Leklem (1981) found that mean intake of 2.0± 0.8 mg/ day of vitamin B-6 resulted in plasma PLP concentration of 51.9± 19.3 nmol/L. Tarr et al (1981) found that mean intake of 2.3 mg/day of vitamin B-6 resulted in plasma PLP concentration of 55.0± 5.7 nmol/L. This lower plasma PLP concentration of this study could be due to overestimation of food portions (especially those high in vitamin B-6 content) by some participants, which would elevate the mean intake of vitamin B-6. A second reason is the slight difference in B-6 to protein ratio, which in this study (Table 2.6) was lower than that reported by Shultz and Leklem (1981) (0.024± 0.007). A third reason is that 45% of the urban and 25% of the rural subjects were cigarette smokers. This high percentage of smokers among the urban and rural groups lowered their mean plasma PLP concentration.

Similarly, the mean red blood cell PLP concentration of both urban and rural groups was lower than that reported by Reynolds et al (1988). This latter study found that mean vitamin B-6 intake of adult males of 2.11± 0.12 mg/day resulted in red blood cell PLP concentration of 85± 6 nmol/L. In contrast, the mean plasma PL and PN concentration of the urban and rural groups were higher than those reported by Driskell et al (2000). This latter study reported that a mean vitamin B-6 intake of 2.87± 1.49 and 2.99± 1.77 for adult men aged 19-24 yr and 25-50 yr respectively, resulted in mean PL and PN concentrations of 7.8± 3.3, 9.3± 4.6 nmol/L and 4.3± 3.2, 5.4± 1.7 nmol/L, respectively. The normal mean plasma alkaline phosphatase activity found in this study for both urban and rural groups suggested no adverse affect of this

enzyme on plasma PLP concentration. The comparison of plasma B-6 vitamers of this study with other studies done in Western males mentioned above indicates that subjects of this study had lower PLP and red blood cell PLP concentrations and higher PL and PN concentrations. These variations could be due to genetic and/or environmental factors such as climate.

The mean urinary 4-PA excretion for both of the urban and rural groups indicated an adequate status based on the suggested value reported by Leklem (1990). Urinary 4-PA was significantly correlated with vitamin B-6 intake, which is in agreement with Shultz and Leklem (1981). However, the mean of the 4-PA excreted, as a percentage of the ingested vitamin B-6, 35.7% and 34.6% for urban and rural groups, respectively was lower than the percentage (40-60%) reported by Wozenski et al (1980) and Leklem (1990). There are two possible reasons for the low excretion of 4-PA in addition to the incomplete urine collection that has been discussed in the previous section. The first is that part of the excretion may have been lost in sweat. This study was performed during the month of August, which is the hottest month in Riyadh; the average highest temperature in Riyadh during the month of August is 113 F° (Statistical Yearbook, 1996). Thus, excretion of the vitamin via sweat could be significant (Johnson et al, 1945). The second reason is the stability of 4-PA in the urine. The 4-PA is relatively stable however, under acidic conditions, the 4-PA can be converted from the lactone to the free acid (Reynolds, 1995). Although the participants of this study were instructed to keep the urine in cool place, with such hot weather, it is likely possible that the preservative evaporated and fermentation

occurred, which could produce an acidic condition. The mean urinary 4-PA to creatinine ratio of the urban and rural groups (Table 2.10) was relatively consistent with results of Bills (1990). This latter study fed nine adult males a constant diet that provided 3.29 mg/day of vitamin B-6 and found a mean urinary 4-PA to creatinine ratio of 4.8± 0.5 μmol/g. In contrast to the relatively low urinary 4-PA excretion, the mean plasma 4-PA concentration of the urban and rural groups was higher relative to values reported by Driskell et al (2000). The latter study reported mean plasma 4-PA concentration of 8.5± 7.2 and 10.9± 5.9 nmol/L for adult males aged 19-24 yr and 25-50 yr, respectively.

Vitamin B-6 status of smokers and non-smokers

The second hypothesis of this study was that smokers, compared to non-smokers have lower plasma B-6 vitamers as well as lower urinary 4-PA excretion. This study found that cigarette smokers, compared to non-smokers had significantly lower mean concentration of plasma PLP. However, the rest of plasma B-6 vitamers, red blood cell PLP concentration and urinary 4-PA excretion were not significantly different between cigarette smokers and non-smokers.

Vitamin B-6 status for the cigarette smokers, water pipe smokers and the non-smokers was adequate based on the mean intake of vitamin B-6, B-6 to protein ratio, plasma PLP concentration and urinary 4-PA excretion. Similar to the findings reported in the urban and rural section, the mean intake of riboflavin, zinc and dietary fiber for

the three groups suggests no adverse affect of these nutrients on vitamin B-6 status. Comparison between cigarette smokers and non-smokers indicated that cigarette smoking lowered plasma PLP concentration. This is in agreement with several studies that reported the adverse effect of cigarette smoking on plasma PLP (Serfontein et al, 1986; Serfontein and Ubbink, 1988; Giraud et al, 1995). The mechanism that explains the effect of cigarette smoking on vitamin B-6 indices is still not well established due to the numerous chemical substances generated by smoking (Cross et al, 1993). Major chemical components that may affect B-6 vitamers are carbon monoxide, cyanide, nicotine and aldehydes (Cross et al, 1993). Cyanide may affect vitamin B-6 metabolism by reacting with PLP to form a cyanohydrine complex, which may cause rapid depletion of plasma PLP (Keniston et al, 1987).

For cigarette smokers, the mean plasma PLP concentration found in this study was lower than that reported by Giraud and Driskell (1994). The latter study reported that young male smokers with an estimated vitamin B-6 intake of 1.86± 0.86 mg/day had mean plasma PLP concentration of 67.4± 12.3 nmol/L. Another study by Giraud et al (1995) reported that intake of vitamin B-6 for adult male smokers of 1.75± 0.79 mg/day corresponded to plasma PLP concentration of 60.9± 19.9 nmol/L. This latter study found that concentrations of plasma PL, PN, 4-PA and red blood cell PLP were 57.9± 46.0, 44.7± 44.8, 33.3± 49.8 and 86.3± 18.4 nmol/L, respectively. The reason for the difference between this study and the above mentioned a study is method of determining vitamin B-6 intake. This study used the three-day food records whereas the two above mentioned studies used 24-hour recall, which provide less details of

dietary intake (Lee and Nieman, 1996). The means of B-6 vitamers concentrations of cigarette smokers reported in this study are consistent with the findings of a controlled study by Sindihebura-Ruhumba (1999). This latter study provided 1.95 mg and 1.65 mg of vitamin B-6 for male and female cigarette smokers, respectively. The mean concentration of PLP, PL, 4-PA, PN and red blood cell PLP were 25.9± 5.0, 17.7± 5.9, 14.2± 1.6, 7.1± 1.7 and 33.9± 5.8 nmol/L, respectively. The normal mean alkaline phosphatase activity for the three groups suggested no adverse affect of the enzyme on plasma PLP concentration. The non-significant difference between cigarette smokers and non-smokers for the plasma concentration of PL, PN and urinary 4-PA excretion found in the present study is consistent with the findings of Sindihebura-Ruhumba (1990). Similarly, the non-significant difference in red blood cell PLP concentration between cigarette smokers and non-smokers reported in this study is consistent with the findings of Giraud et al. (1995) and Vermaak et al. (1990).

The significant differences found in B-6 vitamers between water pipe smokers compared to non-smokers and cigarette smokers were due to the significantly higher intake of vitamin B-6. Another reason, although it was not significant, is the high intake of dietary protein as shown by the lower B-6 to protein ratio in the cigarette smokers and the non-smokers groups compared to the water pipe smokers group. Also, the water pipe smokers compared to the other two groups had lower mean alkaline phosphatase activity. Although the difference was not significant, this low activity may have contributed to the higher mean plasma PLP concentration of the water pipe smokers group. There is no available study, other than this one, that has

investigated the vitamin B-6 status of water pipe smokers. However, in adult male subjects with a B-6 to protein ratio of 0.024 and a vitamin B-6 intake of 2.0 mg/day and 2.3 mg/day corresponded to plasma PLP of 52nmol/L and 55nmol/L, respectively (Leklem, 1990). The mean intake of vitamin B-6 for water pipe smokers was 2.5 mg/day and the B-6 to protein ratio was 0.030 and the mean plasma PLP was 55 nmol/L. This indicates that there was no effect of water pipe smoking on plasma PLP. In addition, there is no study has determined the cyanide in the water pipe smoke. Results of the present study suggest that the water in the water pipe smoking system filters out the cyanide. This study recommends further investigation on the effect of water pipe smoking on vitamin B-6 status.

The mean urinary 4-PA excretion of the three groups indicated adequate vitamin B-6 status based on the suggested value reported by Leklem (1990). The mean of the 4-PA excreted, as a percentage of vitamin B-6 intake was low for cigarette smokers (34.1%) and for non-smokers (33.4%) compared to the percentage (40-60%) reported by Wozenski et al (1980) and Leklem (1990). For water pipe smoking group, the percentage (46.5%) was at the lower end of the range (40-60%). The value of 4-PA excretion was probably low due to factors discussed in the previous section, including incomplete collection of urine, stability of the 4-PA and possible lost of 4-PA in the sweat. However, the mean urinary 4-PA to creatinine ratio for cigarette smokers and non-smokers (Table 2.11) was consistent with the ratios reported by Sindihebura-Ruhumba (1999), 2.8± 0.7 and 3.4± 0.8 for cigarette smokers and non-smokers, respectively. The higher urinary 4-PA to creatinine ratio of water pipe smoking group,

compared to cigarette smokers and non-smokers groups was due to the higher urinary 4-PA excretion. Since this is the first study that examined the affect of water pipe smoking on vitamin B-6 status, comparison of urinary 4-PA excretion with other studies is not possible.

Limitations of the study

There are several limitations of this study that should be considered, first is the small sample size relative to the population of adult males in Riyadh. This was due to the difficulty of recruiting volunteers to participate in such study. In addition, participating in nutritional studies in Saudi Arabia is not common. Another limitation is the seasonal variation in dietary intake. This study was performed during summer season and since certain types of food (especially the fruits) are consumed in higher amounts during the summer months compared to other seasons, different dietary intake may be expected. Also, there were several Saudi foods that do not have a value for the selected micronutrients (vitamin C, vitamin D, vitamin B-3, vitamin B-12, folate, calcium and iron) analyzed in this study, which would underestimate the dietary intakes of these nutrients. In addition, this study did not determine the incomes of the participants, which made classification of the sample, based on the incomes, relative to the Saudi population not possible. This is because asking about income is not appropriate among Saudis and would lower the number of volunteers. Another limitation of this study is that none of the indirect methods of vitamin B-6 status assessment (such as measuring the erythrocyte transaminases activities) was used. In

addition, since cardiovascular disease is prevalent among the adult Saudis, evaluation of homocysteine as related to vitamin B-6 status should have been done.

SUMMARY AND CONCLUSIONS

Nutritional studies in Saudi Arabia are limited and evaluation of the status of specific nutrients for Saudis is needed. Vitamin B-6 status is among the nutritional data needed since no study has yet been done to investigate vitamin B-6 status in any group of the Saudi population. However, two studies found that vitamin B-6 content, compared to protein, was relatively low in fourteen popular meat-based and six popular cereals and legume-based Saudi dishes. Other previous investigations have shown that plasma PLP concentration among cigarette smokers was low compared to non-smokers. Thus, the hypotheses of this study were: 1) vitamin B-6 status, as measured by dietary intake of vitamin B-6, B-6 to protein ratio, plasma B-6 vitamer concentrations and urinary 4-PA excretion, for Saudi adult males in Riyadh region is inadequate. This inadequacy is greater among rural subjects compared to urban subjects. 2) Plasma B-6 vitamer concentrations and urinary 4-PA excretion are low in cigarette smokers compared to non-smokers. In addition, to test the two above-mentioned hypotheses, this study included other objectives:1) determine the intake of nutrients that can affect vitamin B-6 status, including dietary protein, dietary fiber (soluble and insoluble), riboflavin and zinc; 2) Determine the intake of macronutrients and selected micronutrients for urban and rural subjects; 3) Determine the vitamin B-6 content of some Saudi foods as well as some common spices used in Saudi Arabia.

Fifty-one subjects were recruited from urban and rural areas of Riyadh region-Saudi Arabia, 31 and 20, respectively. The subjects were reclassified to non-smokers

(n= 27), cigarette smokers (n= 19) and water pipe smokers (n=5). All participants were instructed to record their dietary intake and collect their 24-hour urine excretion for three consecutive days. On the fourth day, their heights and weights were measured and fasting blood samples were drawn. Hematocrit and hemoglobin were determined from whole blood, the remaining blood samples were centrifuged to separate plasma and red blood cells. Plasma B-6 vitamers (PLP, PL, PN and 4-PA), red blood cell PLP concentrations and urinary 4-PA excretion were determined by HPLC method. Plasma alkaline phosphtase activity and albumin concentration were determined by colorimetric method. Urinary urea nitrogen and creatinine excretion were determined by an automated method. Nutrients intake were determined by the Food Processor as well as other food composition tables. The vitamin B-6 content of Saudi foods and spices was determined by a microbiological assay.

The results showed a high prevalence of overweight and obesity among both urban and rural groups as measured by BMI. Vitamin B-6 content of Saudi foods (halawa tahinea, basbosa, sheariya, dates, molokia, falafel and camel milk) and spices (allspice, black lemon and zatter) indicated that these foods were relatively poor sources of vitamin B-6. Two foods [jarish (whole wheat chunks) and camel meat] and one of the spices (black cumin) had a relatively high content of vitamin B-6. Results of dietary intake showed that the mean intake of saturated fat and dietary cholesterol were high in both urban and rural groups. Based on the recommendations of the DRI, the mean intake of vitamin C, vitamin B-1, vitamin B-3, vitamin B-12 and iron were adequate in both groups. On the other hand, the mean intake of vitamin D, folate and

calcium were lower than the DRI recommendations in the two groups. No significant differences between urban and rural groups were observed in the nutrients intake, except for vitamin D and calcium, which their mean intake, was significantly higher in urban group compared to rural group. The mean dietary intake of nutrients that can affect vitamin B-6 status of urban and rural groups showed a high intake of dietary protein, especially from animal sources. The mean intake of riboflavin was adequate whereas the mean intake of dietary fiber and zinc were lower than the DRI recommendations. No significant differences between the two groups were observed in the dietary intake of these nutrients, except for riboflavin. The mean intake was significantly higher in urban group compared to that of the rural group. Vitamin B-6 status as measured by vitamin B-6 intake, B-6 to protein ratio, plasma PLP concentration and urinary 4-PA excretion was adequate in both urban and rural subjects with no significant difference between the two groups in the mean of these indices. Levels of hematocrit, hemoglobin, alkaline phosphatase activity, albumin concentration, urinary creatinine and urinary urea nitrogen excretion were within the normal ranges. Differences between the two groups in the previous parameters were not statistically significant.

The BMI for non-smokers and smokers indicated a high prevalence of overweight and obesity among the two groups with no significant difference between them. The mean intake of dietary protein was lower in water pipe smokers group compared to cigarette smokers and non-smokers groups, but the difference was not significant. The percentage of vitamin B-6 and protein from animal source was

significantly lower in water pipe smokers group compared to the other two groups. Based on the recommendation of the DRI, the mean intake of riboflavin was adequate in the three groups whereas the mean intake of zinc and dietary fiber were lower than the DRI recommendations. No significant differences were observed between the three groups in the mean intake of these nutrients.

Vitamin B-6 status as measured by vitamin B-6 intake, B-6 to protein ratio, plasma B-6 vitamer concentrations and urinary 4-PA excretion was adequate in the three groups. Compared to cigarette smokers and non-smokers group, water pipe smokers group had significantly higher mean intake of vitamin B-6 as well as higher ratio of B-6 to protein. This high intake of vitamin B-6 resulted in a significant higher concentration of plasma PLP, PL, red blood cells PLP and urinary 4-PA excretion. Concentrations of plasma PN and 4-PA were not significantly different between the three groups. Comparison between non-smokers and cigarette smokers indicated that cigarette smokers compared to non-smokers had a significantly lower plasma PLP concentration. No significant differences were observed between these two groups for the rest of B-6 vitamers. Also, no significant differences were observed between the three groups in plasma alkaline phosphatase activity as well as urinary creatinine excretion. These two measures were within the normal ranges for the three groups. Smokers, compared to non-smokers, had significantly higher values of hematocrit and hemoglobin; both were within the normal ranges. Plasma albumin concentrations and urinary urea nitrogen excretions for smokers and non-smokers were within normal values with no significant differences between the two groups.

In conclusion, these results showed a prevalence of overweight and obesity among adult Saudi males in Riyadh region- Saudi Arabia. These subjects had low intake of dietary fiber, folate and calcium and markedly low intake of vitamin D compared to the DRI recommendations. These subjects also had high intake of saturated fat as well as dietary cholesterol. Vitamin B-6 status of these subjects was adequate with no significant differences between urban and rural groups. Cigarette smokers had higher plasma PLP compared to non-smokers without significant difference in vitamin B-6 intake. This study illustrated that there is a need for increased nutritional education programs and increased physical activity among adult Saudi males to reduce the extent of overweight and obesity. This study recommends fortification of some popular Saudi foods with folate, calcium and vitamin D. In addition, this study indicated the need for changes in Saudi dietary habits to reduce the high intake of saturated fat and dietary cholesterol as well as to increase the intake of dietary fiber. This study also indicated a need for public health programs and governmental policies to reduce the high rate of smoking among adult Saudis. Since different food consumption patterns among different parts of the country of Saudi Arabia were reported, evaluation of dietary intake as well as vitamin B-6 status in other parts of the country is recommended.

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APPENDICES

Key of Abbreviations

- Type of smoking:

  SC: smoking cigarettes

  SW: smoking water pipe

- Physical activity (PA):

  S: Sedentary

  L: light

  M: moderate

  H: hard

- ND: none detected

APPENDIX A. Individual Data (urban subjects)

Table A.1 Descriptive characteristics of the subjects

Subject	Age (y)[1]	Type of Smoking	PA Status
Non-smokers			
U07	40	--	S
U08	36	--	L
U11	26	--	M
U12	30	--	L
U17	40	--	S
U20	29	--	H
U22	41	--	S
U23	35	--	M
U24	23	--	L
U29	43	--	S
U30	20	--	S
U31	26	--	L
Smokers			
U01	31	SC	S
U02	29	SC	S
U04	32	SW	S
U06	28	SC	S
U13	28	SC	S
U14	31	SW	L
U15	37	SC	S
U16	34	SC	S
U18	43	SC	S
U19	27	SW	M
U21	39	SC	L
U25	24	SC	S
U27	38	SW	S
U28	48	SW	S
U32	43	SC	S
U33	39	SC	S
U34	27	SC	S
U35	31	SC	L
U38	32	SC	S

1: The age was reported in Arabic Calendar, year = 354 days

Table A.2 Height, weight and Body Mass Index (BMI)

Subject	Height (cm)	Weight (kg)	BMI (Kg/m^2)
Non-Smokers			
U07	166	73.0	26.5
U08	170	108.0	37.4
U11	174	91.0	30.1
U12	169	103.0	36.7
U17	173	81.0	27.1
U20	175	72.0	23.5
U22	176	74.0	23.9
U23	171	74.5	25.5
U24	166	66.5	24.1
U29	171	76.0	26.0
U30	165	58.0	21.3
U31	169	94.0	32.9
Smokers			
U01	174	71.2	23.5
U02	165	51.6	19.0
U04	170	89.3	30.8
U06	187	82.3	23.5
U13	169	67.0	23.5
U14	168	61.5	21.8
U15	185	87.0	25.4
U16	180	92.0	28.4
U18	177	108.0	34.5
U19	168	56.0	19.8
U21	164	66.0	24.5
U25	178	108.0	34.1
U27	167	90.0	32.3
U28	174	64.5	21.3
U32	165	60.0	22.0
U33	165	64.5	23.7
U34	173	124.0	41.4
U35	172	81.5	27.6
U38	175	86.0	28.1

Table A.3 Hemoglobin and hematocrit

Subject	Hemoglobin (g/L)	Hematocrit (%)
Non-Smokers		
U07	144	42
U08	158	47
U11	162	48
U12	159	47
U17	153	44
U20	166	50
U22	154	46
U23	155	47
U24	175	52
U29	154	47
U30	177	52
U31	156	47
Smokers		
U01	154	47
U02	151	44
U04	162	49
U06	168	52
U13	162	48
U14	161	48
U15	176	52
U16	158	47
U18	176	54
U19	174	51
U21	157	46
U25	175	52
U27	167	49
U28	166	48
U32	158	47
U33	151	45
U34	159	47
U35	173	52
U38	170	51

Table A.4 Plasma B-6 vitamers and red blood cell (RBC) PLP concentration

Subject	Plasma PLP (nmol/L)	Plasma 4-PA (nmol/L)	Plasma PL (nmol/L)	Plasma PN (nmol/L)	RBC PLP (nmol/L)
Non-Smokers					
U07	26.9	7.7	7.9	9.8	33.3
U08	45.8	18.8	15.6	8.1	46.6
U11	34.0	13.2	14.9	6.4	--
U12	22.4	12.0	16.0	9.5	21.5
U17	35.8	14.2	12.9	7.7	29.6
U20	34.5	19.8	6.3	6.5	23.7
U22	61.4	14.1	15.0	7.1	44.3
U23	34.4	12.6	10.8	6.0	30.2
U24	40.9	12.1	13.6	5.0	32.0
U29	31.0	18.5	9.2	15.9	24.3
U30	42.6	11.1	17.4	15.7	34.1
U31	44.3	12.7	6.4	14.1	23.3
Smokers					
U01	34.6	16.0	ND	6.8	23.2
U02	39.4	16.0	21.1	7.1	26.6
U04	52.2	17.3	11.3	10.3	41.0
U06	71.8	17.3	19.7	10.2	35.0
U13	22.3	7.6	11.7	9.3	31.6
U14	94.6	13.6	27.7	6.8	38.0
U15	11.8	8.7	9.4	7.1	15.6
U16	41.2	11.6	10.9	9.2	33.8
U18	18.2	12.9	10.5	7.0	26.3
U19	32.2	11.9	ND	6.2	19.6
U21	21.5	11.1	14.0	8.1	20.0
U25	34.6	7.4	11.7	5.3	35.0
U27	49.4	13.7	14.9	5.6	32.2
U28	63.5	31.9	32.3	10.3	37.8
U32	42.2	13.2	9.8	6.8	39.6
U33	66.9	10.5	24.8	16.2	35.9
U34	17.2	8.4	6.9	14.9	25.4
U35	29.2	11.4	ND	ND	20.1
U38	23.0	11.7	9.9	5.7	17.2

Table A.5 Plasma alkaline phosphatase activity and albumin concentration

Subject	Alkaline phosphatase (U/L)	Albumin (g./L)
Non-Smokers		
U07	13.4	50
U08	27.8	47
U11	25.5	48
U12	19.5	49
U17	31.6	48
U20	23.2	51
U22	29.9	50
U23	27.0	51
U24	20.4	56
U29	32.8	57
U30	50.0	58
U31	18.0	49
Smokers		
U01	23.3	53
U02	14.4	49
U04	20.2	51
U06	23.1	56
U13	33.9	54
U14	13.6	54
U15	17.5	52
U16	23.9	51
U18	25.7	56
U19	19.4	53
U21	24.6	50
U25	46.1	54
U27	23.7	50
U28	31.3	51
U32	20.3	51
U33	31.2	53
U34	30.8	52
U35	23.8	52
U38	37.5	53

Table A.6. Urinary 4-PA, creatinine and urea nitrogen excretion and 4-PA/creatinine ratio

Subject	Day #	4-PA (μmol/ day)	Creatinine (g/day)	4-PA/ Creatinine ratio	Urea N. (g/day)
Non-smokers					
U07	1	3.1	1.5	2.1	11.0
	2	3.4	1.7	2.1	13.8
	3	2.1	1.2	1.7	9.2
	Avg.	2.9	1.5	2.0	11.3
U08	1	6.2	1.4	4.6	8.7
	2	5.1	1.2	4.2	7.9
	3	7.1	1.8	4.0	10.0
	Avg.	6.1	1.5	4.3	8.9
U11	1	3.1	0.9	3.6	4.8
	2	5.0	0.8	6.4	5.5
	3	4.8	1.3	3.7	10.8
	Avg.	4.3	1.0	4.6	7.0
U12	1	4.3	1.7	2.6	8.8
	2	3.4	1.3	2.6	5.6
	3	6.2	2.3	2.7	13.2
	Avg.	4.6	1.8	2.6	9.2
U17	1	3.7	1.6	2.4	4.8
	2	3.6	1.3	2.9	4.9
	3	4.0	1.6	2.6	7.9
	Avg.	3.8	1.5	2.6	5.9
U20	1	3.5	1.2	2.9	8.2
	2	6.7	2.3	2.9	14.3
	3	5.2	1.6	3.3	8.3
	Avg.	5.1	1.7	3.0	10.3
U22	1	5.1	1.7	3.1	10.8
	2	5.3	1.4	3.7	8.6
	3	5.1	1.9	2.6	13.3
	Avg.	5.2	1.7	3.1	10.9

Table A.6. (cont.)

U23	1	6.5	2.0	3.4	10.4
	2	6.5	2.1	3.0	11.6
	3	5.5	1.9	2.9	9.3
	Avg.	6.2	2.0	3.1	10.4
U24	1	4.5	1.4	3.3	9.3
	2	3.7	1.2	3.2	6.8
	3	3.5	1.1	3.4	7.8
	Avg.	3.9	1.2	3.3	8.0
U29	1	6.0	1.9	3.1	9.1
	2	4.8	1.6	3.0	7.4
	3	4.8	1.8	2.7	9.0
	Avg.	5.2	1.8	2.9	8.5
U30	1	2.7	1.1	2.4	7.6
	2	2.6	1.0	2.5	6.9
	3	3.2	1.2	2.8	8.6
	Avg.	2.8	1.1	2.6	7.7
U31	1	4.5	1.3	3.6	6.4
	2	4.1	1.3	3.1	10.1
	3	6.3	1.8	3.4	10.9
	Avg.	5.0	1.5	3.4	9.1
Smokers					
U01	1	3.9	1.6	2.4	5.7
	2	1.7	1.0	1.7	8.1
	3	1.5	1.0	1.5	5.3
	Avg.	2.4	1.2	1.9	6.4
U02	1	0.9	0.9	1.0	4.5
	2	2.0	0.7	3.0	4.3
	3	0.2	0.4	0.6	2.9
	Avg.	1.0	0.7	1.5	3.9
U04	1	5.3	1.4	3.8	13.8
	2	6.2	1.1	5.6	14.5
	3	3.5	1.8	2.0	9.4
	Avg.	5.0	1.4	3.8	12.6

Table A.6. (cont.)

U06	1	5.9	1.7	3.4	11.6
	2	6.8	2.2	3.1	14.9
	3	5.2	1.0	5.0	6.2
	Avg.	6.0	1.6	3.8	10.9
U13	1	5.4	1.4	3.8	11.8
	2	4.0	1.4	2.8	10.5
	3	4.1	1.7	2.5	12.4
	Avg.	4.5	1.5	3.0	11.6
U14	1	7.6	1.7	4.4	7.6
	2	4.3	1.2	3.7	4.8
	3	3.5	1.1	3.1	4.7
	Avg.	5.1	1.3	3.7	5.7
U15	1	3.9	1.7	2.3	5.5
	2	2.7	1.4	1.9	4.7
	3	2.0	1.4	1.4	3.7
	Avg.	2.9	1.5	1.9	4.6
U16	1	2.8	1.4	1.9	8.1
	2	4.0	2.5	1.6	15.2
	3	4.2	1.2	3.5	6.5
	Avg.	3.7	1.7	2.3	9.9
U18	1	4.6	1.7	2.8	9.6
	2	4.8	1.6	3.0	10.5
	3	5.1	1.7	3.0	9.3
	Avg.	4.8	1.7	2.9	9.8
U19	1	3.7	1.3	3.0	6.6
	2	3.9	1.4	2.8	9.3
	3	4.0	1.4	2.9	6.0
	Avg.	3.9	1.4	2.9	7.3
U21	1	3.2	1.2	2.7	6.2
	2	4.3	1.9	2.3	9.2
	3	3.1	1.4	2.2	6.1
	Avg.	3.5	1.5	2.4	7.2

Table A.6. (cont.)

U25	1	6.1	2.5	2.5	10.8
	2	4.8	2.1	2.4	8.5
	3	5.3	2.5	2.1	11.2
	Avg.	5.4	2.4	2.3	10.2
U27	1	5.6	2.0	2.8	12.2
	2	5.1	1.6	3.3	8.7
	3	5.3	1.9	2.8	9.4
	Avg.	5.3	1.8	3.0	10.1
U28	1	19.7	1.6	12.5	5.2
	2	13.2	0.9	14.2	3.1
	3	12.9	1.3	9.8	3.0
	Avg.	15.3	1.3	12.2	3.8
U32	1	3.5	1.3	2.8	8.1
	2	4.3	1.6	2.6	10.0
	3	3.1	1.2	2.5	8.0
	Avg.	3.6	1.4	2.6	8.7
U33	1	2.8	1.1	2.6	7.2
	2	5.8	1.1	5.4	5.5
	3	4.5	1.3	3.5	6.9
	Avg.	4.4	1.2	3.8	6.5
U34	1	4.7	1.2	3.8	12.9
	2	3.7	2.0	1.8	12.5
	3	3.4	1.7	2.0	9.7
	Avg.	3.9	1.6	2.5	11.7
U35	1	3.6	2.0	1.8	9.9
	2	4.5	2.0	2.2	12.4
	3	3.5	1.5	2.3	9.2
	Avg.	3.9	1.8	2.1	10.5
U38	1	2.7	1.5	1.9	10.0
	2	2.3	1.4	1.7	9.2
	3	3.4	1.6	2.2	9.3
	Avg.	2.8	1.5	1.9	9.5

Table A.7. Intake of vitamin B-6, dietary protein, B-6:protein ratio, B-6 and protein from animal source

Subject	Day #	Vitamin B-6 (mg)	Protein (g)	B6:protein ratio(mg/g)	B-6 from animal source (%)	Protein from animal source (%)
Non-smokers						
U07	1	1.44	55	0.026	30.0	57.0
	2	1.12	79	0.014	32.1	45.0
	3	1.80	79	0.023	29.6	50.0
	Avg.	1.45	71	0.021	30.6	44.8
U08	1	2.42	106	0.023	61.0	70.0
	2	2.46	159	0.015	54.0	75.0
	3	2.37	120	0.020	56.0	66.0
	Avg.	2.42	128	0.019	57.0	70.3
U11	1	1.98	80	0.025	46.0	73.0
	2	3.00	147	0.020	70.0	73.0
	3	2.48	144	0.017	67.0	65.0
	Avg.	2.49	124	0.021	61.0	70.3
U12	1	3.39	131	0.026	27.6	53.2
	2	2.97	125	0.024	32.7	60.0
	3	2.85	127	0.022	28.6	49.4
	Avg.	3.07	128	0.024	29.6	54.2
U17	1	2.10	74	0.028	32.0	45.0
	2	2.23	68	0.030	47.0	61.0
	3	1.91	80	0.024	56.0	64.0
	Avg.	2.08	74	0.027	45.0	56.7
U20	1	3.03	102	0.021	63.0	60.0
	2	2.46	137	0.018	58.0	65.0
	3	2.74	143	0.019	74.0	85.0
	Avg.	2.74	127	0.019	65.0	70.0
U22	1	2.00	84	0.024	56.0	77.0
	2	1.59	77	0.021	53.0	56.0
	3	2.29	96	0.024	72.0	79.0
	Avg.	1.96	86	0.023	60.3	70.7

Table A.7. (cont.)

U23	1	1.63	78	0.021	51.0	72.0
	2	2.77	111	0.025	46.0	73.0
	3	1.44	73	0.020	48.0	71.0
	Avg.	1.95	87	0.022	48.3	72.0
U24	1	1.52	70	0.021	7.0	21.0
	2	1.68	101	0.017	53.0	59.0
	3	1.59	88	0.018	68.0	65.0
	Avg.	1.60	86	0.019	42.7	48.3
U29	1	1.65	83	0.021	56.0	75.0
	2	1.21	78	0.015	40.0	58.0
	3	1.41	89	0.016	51.0	62.0
	Avg.	1.42	83	0.017	49.0	65.0
U30	1	2.74	138	0.020	57.0	74.0
	2	2.31	127	0.018	51.0	65.0
	3	1.76	114	0.015	48.0	61.0
	Avg.	2.27	126	0.018	52.0	66.7
U31	1	2.64	166	0.016	51.0	65.0
	2	2.41	116	0.021	49.0	62.0
	3	1.91	110	0.017	50.0	59.0
	Avg.	2.32	131	0.018	50.0	62.0
Smokers						
U01	1	0.93	55	0.017	25.0	51.0
	2	1.43	84	0.017	42.0	58.0
	3	2.92	70	0.042	25.0	70.0
	Avg.	1.76	70	0.025	30.7	59.7
U02	1	1.26	80	0.016	53.0	55.0
	2	1.87	92	0.020	64.0	64.0
	3	1.67	101	0.017	78.0	69.0
	Avg.	1.60	91	0.018	65.0	62.7
U04	1	1.94	75	0.026	39.0	59.0
	2	1.60	76	0.021	51.0	61.0
	3	1.39	80	0.017	55.0	61.0
	Avg.	1.64	77	0.021	48.3	60.3

Table A.7. (cont.)

U06	1	1.97	87	0.023	40.0	53.0
	2	2.84	98	0.029	45.0	68.0
	3	2.64	97	0.027	48.0	71.0
	Avg.	2.48	94	0.026	44.3	64.0
U13	1	1.70	109	0.016	71.0	70.0
	2	1.65	72	0.023	72.0	71.0
	3	1.41	99	0.014	70.0	68.0
	Avg.	1.59	93	0.018	71.0	69.7
U14	1	1.31	92	0.014	59.0	59.0
	2	2.30	60	0.038	15.0	38.0
	3	2.18	88	0.025	67.0	75.0
	Avg.	1.93	80	0.026	47.0	57.3
U15	1	2.54	161	0.016	61.0	51.0
	2	1.43	82	0.017	48.0	55.0
	3	1.12	88	0.012	46.0	49.0
	Avg.	1.70	110	0.015	51.7	51.7
U16	1	2.17	102	0.021	49.0	56.0
	2	4.35	180	0.024	45.0	80.0
	3	2.52	116	0.022	32.0	76.0
	Avg.	3.01	133	0.022	42.0	70.7
U18	1	1.19	81	0.014	76.0	48.0
	2	3.29	145	0.022	50.0	77.0
	3	1.07	91	0.012	48.0	51.0
	Avg.	1.85	106	0.016	58.0	58.7
U19	1	4.28	178	0.024	68.0	82.0
	2	3.95	124	0.032	31.0	57.0
	3	2.13	120	0.017	52.0	51.0
	Avg.	3.45	141	0.024	50.3	63.3
U21	1	0.90	90	0.010	78.0	70.0
	2	3.59	123	0.029	48.0	67.0
	3	1.12	93	0.012	49.0	65.0
	Avg.	1.87	102	0.017	58.3	67.3

Table A.7. (cont.)

U25	1	5.17	161	0.032	64.0	84.0
	2	5.30	88	0.060	16.0	57.0
	3	1.23	92	0.013	52.0	56.0
	Avg.	3.90	114	0.035	44.0	65.7
U27	1	2.15	99	0.022	51.0	65.0
	2	4.17	161	0.026	48.0	77.0
	3	1.68	90	0.019	43.0	82.0
	Avg.	2.67	117	0.022	47.3	74.7
U28	1	2.81	49	0.057	5.0	25.0
	2	3.06	50	0.061	5.0	27.0
	3	2.76	49	0.057	5.0	24.0
	Avg.	2.88	49	0.058	5.0	25.3
U32	1	1.53	95	0.016	62.0.	76.0
	2	2.69	119	0.022	65.0	82.0
	3	1.46	92	0.016	63.0	78.0
	Avg.	1.89	102	0.018	63.3	78.7
U33	1	1.81	99	0.018	62.0	64.0
	2	2.27	84	0.027	15.0	42.0
	3	1.92	89	0.022	59.0	59.0
	Avg.	2.00	91	0.022	45.3	55.0
U34	1	1.21	124	0.010	41.0	55.0
	2	1.35	119	0.011	48.0	50.0
	3	2.07	99	0.021	38.0	47.0
	Avg.	1.54	114	0.014	42.3	50.7
U35	1	1.79	85	0.021	43.0	56.0
	2	2.91	110	0.027	52.0	61.0
	3	2.73	104	0.026	54.0	67.0
	Avg.	2.48	100	0.025	49.7	61.3
U38	1	1.23	81	0.015	52.0	75.0
	2	2.31	98	0.024	56.0	77.0
	3	1.14	102	0.011	48.0	63.0
	Avg.	1.56	94	0.016	52.0	71.7

Table A.8. Intake of soluble dietary fiber (SDF), insoluble dietary fiber (IDF), riboflavin and zinc

Subject	Day #	SDF (g)	IDF (g)	Riboflavin (mg)	Zn (mg)
Non-smokers					
U07	1	6.1	18.2	1.00	6.6
	2	5.9	6.3	1.44	13.1
	3	2.6	7.8	2.33	15.7
	Avg.	4.9	10.8	1.59	11.8
U08	1	5.0	12.8	2.15	11.0
	2	4.5	14.4	2.59	13.2
	3	5.0	13.5	2.59	11.9
	Avg.	4.8	13.6	2.44	12.0
U11	1	2.4	7.3	1.29	6.3
	2	4.9	12.6	2.18	8.9
	3	4.6	8.4	2.51	10.0
	Avg.	4.0	9.4	1.99	8.4
U12	1	3.8	4.9	2.88	10.2
	2	2.4	5.9	2.55	11.1
	3	3.2	5.6	2.67	9.8
	Avg.	3.1	5.5	2.70	10.4
U17	1	8.1	21.5	1.10	9.7
	2	3.3	8.1	3.52	8.6
	3	5.6	7.9	2.21	8.9
	Avg.	5.7	12.5	2.28	9.1
U20	1	2.3	5.7	1.96	8.9
	2	3.3	7.1	2.09	9.3
	3	3.1	8.5	3.50	11.9
	Avg.	2.9	7.1	3.52	10.0
U22	1	3.2	7.8	1.83	11.6
	2	1.8	4.0	1.68	22.4
	3	3.4	7.0	1.78	10.2
	Avg.	2.8	6.3	1.76	14.7

Table A.8. (cont.)

U23	1	3.2	10.8	2.24	7.7
	2	2.7	8.3	2.51	11.6
	3	2.7	7.8	2.13	6.5
	Avg.	2.9	9.0	2.29	8.6
U24	1	9.4	23.8	2.03	6.7
	2	5.1	8.6	1.89	7.3
	3	3.2	6.8	1.94	6.9
	Avg.	5.9	13.1	1.95	7.0
U29	1	3.1	7.7	1.64	7.7
	2	1.7	4.8	1.45	6.9
	3	2.9	5.7	1.51	7.7
	Avg.	2.6	6.1	1.53	7.4
U30	1	2.2	5.2	1.69	7.9
	2	3.1	6.4	1.97	8.4
	3	2.9	7.5	2.78	9.9
	Avg.	2.7	6.4	2.15	8.7
U31	1	2.8	5.2	1.83	7.6
	2	2.4	6.2	2.64	10.1
	3	3.1	7.8	2.49	9.8
	Avg.	2.8	6.4	2.32	9.2
Smokers					
U01	1	2.2	4.3	1.24	4.3
	2	9.6	11.1	1.40	8.5
	3	1.6	7.1	6.28	11.9
	Avg.	4.5	7.5	2.97	8.2
U02	1	5.2	7.1	1.22	7.7
	2	3.0	7.3	1.45	6.2
	3	2.7	5.9	2.34	8.4
	Avg.	3.6	6.8	1.67	7.4
U04	1	4.2	8.2	1.85	8.6
	2	0.7	1.9	1.25	8.9
	3	0.5	1.9	1.20	10.1
	Avg.	1.8	4.0	1.43	9.2

Table A.8. (cont.)

U06	1	3.4	7.0	5.63	13.5
	2	2.3	5.3	2.30	15.3
	3	4.8	4.9	2.11	10.5
	Avg.	3.5	5.7	3.35	13.1
U13	1	1.0	2.0	2.03	11.7
	2	1.2	3.8	1.54	9.8
	3	2.1	3.6	1.83	10.2
	Avg.	1.4	3.1	1.80	10.6
U14	1	3.5	8.6	2.29	8.2
	2	2.3	8.1	0.92	7.2
	3	3.0	7.8	2.79	7.8
	Avg.	2.9	8.2	2.00	7.7
U15	1	2.6	6.5	0.96	6.5
	2	2.0	5.4	2.14	18.9
	3	2.4	6.5	2.08	10.1
	Avg.	2.3	6.1	1.73	11.8
U16	1	2.0	6.0	1.54	7.1
	2	7.3	9.3	2.09	11.6
	3	5.1	8.6	1.94	11.1
	Avg.	4.8	8.0	1.86	9.9
U18	1	2.5	4.2	2.73	6.2
	2	9.0	22.5	3.05	12.1
	3	2.7	6.4	2.81	8.6
	Avg.	4.7	11.0	2.86	9.0
U19	1	2.8	5.8	5.79	21.4
	2	13.2	27.4	2.05	20.2
	3	3.8	7.3	1.97	19.6
	Avg.	6.6	13.5	3.27	20.4
U21	1	2.1	6.0	2.23	11.6
	2	5.5	13.9	8.16	22.4
	3	3.6	9.7	7.61	10.2
	Avg.	3.7	9.9	6.00	14.7

Table A.8. (cont.)

U25	1	5.2	14.4	2.03	22.3
	2	16.1	22.4	2.44	13.9
	3	4.1	9.0	1.93	8.2
	Avg.	8.5	15.3	2.13	14.8
U27	1	2.1	6.9	1.32	6.8
	2	3.1	7.7	1.82	9.4
	3	4.6	10.2	1.94	7.7
	Avg.	3.3	8.3	1.69	8.0
U28	1	5.6	20.7	2.42	6.1
	2	4.8	19.7	2.13	5.8
	3	5.3	18.7	2.03	5.9
	Avg.	5.2	19.7	2.19	5.9
U32	1	3.8	8.8	1.77	11.9
	2	2.8	6.3	1.91	10.3
	3	3.3	7.4	1.86	9.8
	Avg.	3.3	7.5	1.85	10.7
U33	1	5.5	10.2	3.26	12.3
	2	5.2	17.8	2.68	35.7
	3	4.8	13.4	2.67	11.8
	Avg.	5.2	13.8	2.87	19.9
U34	1	2.0	4.9	1.43	9.2
	2	6.7	10.5	1.41	8.6
	3	4.8	9.8	0.93	11.6
	Avg.	4.5	8.4	1.26	9.8
U35	1	2.5	5.9	4.43	11.9
	2	3.2	5.6	3.31	9.9
	3	3.8	7.7	2.79	10.1
	Avg.	3.2	6.4	3.51	10.6
U38	1	3.1	7.1	1.31	8.8
	2	3.9	8.2	1.98	9.9
	3	4.5	11.9	1.87	11.1
	Avg.	3.8	9.1	1.72	9.9

Table A.9. Intake of calories, carbohydrates, fat, saturated fat (S-fat), and unsaturated fat (U-fat)

Subject	Day #	Calories (K.cal)	Carbohy-drates (g)	Fat (g)	S-Fat (g)	U-Fat (g)
Non-smokers						
U03	1	2440	341	47	13	30
	2	2561	359	71	28	37
	3	2842	469	57	26	24
	Avg.	2614	390	58	22	30
U07	1	2118	325	64	18	38
	2	2217	414	27	8	9
	3	2109	232	103	24	68
	Avg.	2148	324	65	17	38
U08	1	2981	367	118	47	59
	2	3284	334	151	66	74
	3	3095	350	140	61	69
	Avg.	3120	350	136	58	67
U11	1	3010	399	125	33	72
	2	3134	430	96	23	57
	3	3150	446	88	41	36
	Avg.	3098	425	103	32	55
U12	1	3163	416	109	28	75
	2	2992	454	76	18	53
	3	3088	514	60	17	38
	Avg.	3081	461	82	21	55
U17	1	2131	384	45	8	34
	2	2261	340	78	13	56
	3	2198	334	62	11	45
	Avg.	2197	353	62	11	45
U20	1	2067	330	41	18	19
	2	1791	171	66	21	40
	3	2256	274	67	27	32
	Avg.	2038	258	58	22	30

Table A.9. (cont.)

U22	1	2076	309	61	24	29
	2	2044	160	129	23	99
	3	2055	207	96	27	66
	Avg.	2058	225	95	25	65
U23	1	3348	535	113	46	59
	2	2700	392	81	20	54
	3	3019	464	95	33	56
	Avg.	3022	464	96	33	56
U24	1	2632	379	97	35	56
	2	2648	298	120	67	44
	3	2711	342	111	52	54
	Avg.	2664	340	109	51	51
U29	1	2072	328	57	19	33
	2	2085	313	81	37	19
	3	2076	280	69	26	25
	Avg.	2078	307	69	27	26
U30	1	1933	211	58	21	32
	2	1990	202	74	20	37
	3	1950	239	59	16	19
	Avg.	1958	217	64	19	29
U31	1	3005	286	134	62	51
	2	3060	398	111	50	41
	3	3115	477	89	39	31
	Avg.	3060	387	111	50	41
Smokers						
U01	1	2038	311	66	31	14
	2	2295	315	79	26	47
	3	2136	317	67	18	44
	Avg.	2156	314	71	25	35
U02	1	1907	250	65	36	26
	2	1861	265	46	10	21
	3	1949	248	62	16	36
	Avg.	1906	254	58	21	28

Table A.9. (cont.)

U04	1	2494	248	140	27	106
	2	2420	314	93	33	31
	3	2410	276	108	35	35
	Avg.	2441	279	114	32	57
U06	1	2343	335	77	14	57
	2	2333	292	89	24	59
	3	2495	338	83	18	57
	Avg.	2390	322	83	19	58
U13	1	2131	250	78	18	52
	2	2095	251	90	24	48
	3	2418	307	86	28	51
	Avg.	2215	269	85	23	50
U14	1	2418	323	83	33	44
	2	2552	357	101	26	69
	3	2209	296	76	28	42
	Avg.	2393	325	87	29	52
U15	1	2243	316	47	21	13
	2	2158	297	75	24	46
	3	2387	266	113	28	75
	Avg.	2263	293	78	24	45
U16	1	2407	338	79	25	48
	2	2538	317	63	14	39
	3	2401	257	107	41	55
	Avg.	2449	304	83	27	47
U18	1	2557	237	137	55	58
	2	2599	308	90	31	53
	3	2582	303	113	46	62
	Avg.	2579	283	113	44	58
U19	1	2545	275	83	25	49
	2	2604	335	89	23	55
	3	2570	330	86	25	51
	Avg.	2573	313	86	24	52

Table A.9. (cont.)

U21	1	2319	205	130	44	74
	2	2461	235	117	32	72
	3	2393	221	125	39	71
	Avg.	2391	220	124	38	72
U25	1	2979	463	58	18	33
	2	2915	526	66	19	37
	3	2948	500	64	19	36
	Avg.	2947	496	63	19	35
U27	1	2511	415	49	15	29
	2	2470	346	47	14	29
	3	2366	331	78	24	41
	Avg.	2449	364	58	18	33
U28	1	1809	380	17	5	11
	2	1806	396	7	2	4
	3	1842	373	18	5	11
	Avg.	1819	383	14	4	9
U32	1	1923	247	61	18	25
	2	1816	194	64	15	44
	3	1871	233	63	17	39
	Avg.	1870	225	63	17	36
U33	1	2423	305	94	26	58
	2	2198	253	100	24	62
	3	2309	275	97	25	60
	Avg.	2310	278	97	25	60
U34	1	3142	436	103	46	41
	2	3101	471	98	20	43
	3	3180	462	108	48	35
	Avg.	3141	456	103	38	40
U35	1	2965	312	162	70	81
	2	2673	211	158	45	99
	3	2952	405	108	38	53
	Avg.	2863	309	143	51	78
U38	1	2412	328	86	20	56
	2	2401	307	89	15	42
	3	2425	352	84	17	60
	Avg.	2413	329	86	17	53

Table A.10. Intake of vitamin C, vitamin B1, vitamin D, calcium and iron

Subject	Day #	Vitamin C (mg)	Vitamin B1 (mg)	Vitamin D (IU)	Calcium (mg)	Iron (mg)
Non-smokers						
U03	1	37	1.3	47	931	11.8
	2	216	2.1	36	1588	17.5
	3	98	0.8	50	971	12.2
	Avg.	117	1.4	44	1163	13.8
U07	1	41	0.6	112	647	13.2
	2	51	0.7	9	546	9.0
	3	23	1.2	5	794	10.3
	Avg.	38	0.8	42	662	10.8
U08	1	225	2.0	21	646	21.5
	2	224	2.2	387	921	14.0
	3	227	2.3	29	988	9.9
	Avg.	225	2.2	146	852	15.1
U11	1	113	2.1	29	655	9.5
	2	51	2.4	62	661	15.0
	3	23	3.1	456	659	6.3
	Avg.	62	2.5	182	658	10.3
U12	1	59	1.7	90	1954	13.5
	2	41	2.1	41	1670	15.4
	3	230	2.2	22	859	9.5
	Avg.	110	2.0	51	1494	12.8
U17	1	187	1.3	22	472	13.8
	2	695	1.3	19	313	14.1
	3	512	1.2	40	452	12.7
	Avg.	465	1.3	27	412	13.5
U20	1	299	1.1	52	1148	9.3
	2	81	0.8	24	700	7.7
	3	54	1.6	58	2012	11.8
	Avg.	145	1.2	45	1287	9.6

Table A.10. (cont.)

U22						
	1	246	0.9	26	980	12.1
	2	184	1.4	12	823	8.1
	3	114	1.1	40	971	9.3
	Avg.	181	1.1	26	925	9.8
U23	1	222	2.1	48	1616	18.8
	2	245	1.0	33	1703	7.6
	3	186	1.2	42	1312	9.8
	Avg.	218	1.4	41	1544	12.1
U24	1	46	2.7	8	662	8.8
	2	51	2.1	35	632	12.5
	3	49	1.9	30	666	9.6
	Avg.	49	2.2	24	653	10.3
U29	1	142	2.2	19	410	10.8
	2	24	0.6	36	1837	7.2
	3	94	1.8	41	818	8.3
	Avg.	87	1.5	32	1022	8.8
U30	1	16	0.9	41	231	9.7
	2	97	1.1	36	695	7.6
	3	16	1.1	48	1378	8.6
	Avg.	43	1.0	42	768	8.6
U31	1	29	1.7	183	3411	8.8
	2	91	1.8	32	4197	9.1
	3	86	1.3	47	2812	8.9
	Avg.	69	1.6	87	3473	8.9
Smokers						
U01	1	120	1.2	10	810	7.4
	2	210	1.7	7	771	9.4
	3	163	2.5	168	1015	40.7
	Avg.	164	1.8	62	865	19.2
U02	1	118	1.4	17	513	9.1
	2	313	1.8	18	408	12.9
	3	126	1.6	63	849	15.8
	Avg.	186	1.6	33	590	12.6
U04	1	52	1.3	50	1127	9.8
	2	165	0.7	16	635	9.4
	3	107	1.4	4	586	11.4
	Avg.	108	1.1	23	783	10.2

Table A.10.(cont)

U06	1	386	2.7	37	777	16.4
	2	155	1.7	23	794	12.2
	3	167	1.5	22	812	10.8
	Avg.	236	2.0	27	794	13.1
U13	1	101	1.5	46	858	12.1
	2	13	0.8	2	640	11.6
	3	258	1.0	45	698	9.1
	Avg.	124	1.1	31	732	10.9
U14	1	40	1.9	41	1056	10.4
	2	109	1.2	11	352	8.9
	3	56	2.3	18	888	11.7
	Avg.	68	1.8	23	765	10.3
U15	1	93	0.9	34	1134	11.3
	2	179	1.2	16	365	8.4
	3	196	1.3	9	861	16.9
	Avg.	156	1.1	20	787	12.2
U16	1	235	1.5	37	590	12.1
	2	288	2.6	502	582	15.2
	3	230	1.6	363	573	13.8
	Avg.	251	1.9	301	582	13.7
U18	1	42	1.2	4	1695	11.8
	2	99	1.9	121	1724	22.3
	3	115	1.2	7	1584	9.7
	Avg.	85	1.4	44	1668	14.6
U19	1	266	1.6	42	733	22.7
	2	167	2.6	0	474	18.7
	3	120	1.8	19	614	9.3
	Avg.	184	2.0	20	607	16.9
U21	1	25	1.4	71	888	11.5
	2	327	2.2	30	497	25.9
	3	188	1.6	46	633	10.7
	Avg.	180	1.7	49	673	16.0

Table A.10. (cont.)

U25	1	61	1.0	23	591	13.7
	2	382	2.8	7	965	15.8
	3	118	1.2	14	835	12.2
	Avg.	187	1.7	15	797	13.9
U27	1	142	1.2	65	698	10.2
	2	126	1.3	9	675	7.6
	3	112	1.1	19	681	8.7
	Avg.	127	1.2	31	685	8.8
U28	1	160	2.2	148	664	39.6
	2	241	2.3	215	327	50.6
	3	192	2.2	171	571	41.2
	Avg.	198	2.2	178	521	43.8
U32	1	58	1.2	24	829	9.4
	2	149	1.3	41	1056	8.2
	3	98	1.2	44	914	7.8
	Avg.	102	1.2	36	933	8.5
U33	1	96	2.0	24	1527	12.3
	2	197	2.1	115	1330	29.2
	3	121	1.9	37	1413	11.2
	Avg.	138	2.0	59	1423	17.6
U34	1	303	2.0	51	737	18.0
	2	109	1.3	10	929	12.1
	3	97	1.7	14	873	10.8
	Avg.	170	1.7	25	846	13.6
U35	1	13	0.8	89	908	10.1
	2	254	0.8	8	1824	17.8
	3	116	2.0	20	1176	9.9
	Avg.	128	1.2	39	1303	12.6
U38	1	36	0.9	81	829	14.8
	2	144	2.0	15	957	14.5
	3	97	1.3	19	887	13.8
	Avg.	92	1.4	38	891	14.4

Table A.11. Intake of cholesterol, vitamin B3, vitamin B12 and folate

Subject	Day #	Cholesterol (mg)	Vitamin B3 (mg)	Vitamin B12 (µg)	Folate (µg)
Non-smokers					
U03	1	312	52.5	3.5	128
	2	254	39.6	3.5	218
	3	317	39.2	1.9	202
	Avg.	294	43.8	3.0	183
U07	1	462	49.7	5.4	42
	2	103	15.1	0.6	105
	3	115	15.0	3.8	112
	Avg.	227	26.6	3.3	86
U08	1	954	39.4	7.1	209
	2	446	52.5	8.1	136
	3	406	37.2	5.2	135
	Avg.	602	43.0	6.8	160
U11	1	206	40.8	1.7	88
	2	704	60.9	3.1	249
	3	404	63.7	1.7	190
	Avg.	438	55.1	2.2	176
U12	1	237	39.9	3.7	296
	2	221	43.3	3.0	151
	3	167	36.6	2.4	268
	Avg.	208	39.9	3.0	238
U17	1	124	27.6	0.9	249
	2	373	27.8	52.5	419
	3	248	27.2	1.3	229
	Avg.	248	27.5	18.2	299
U20	1	347	40.7	4.8	168
	2	282	33.6	2.2	116
	3	683	37.2	5.8	189
	Avg.	437	37.2	4.3	158

Table A.11. (cont.)

U22	1	562	19.2	3.4	156
	2	114	21.5	1.7	167
	3	347	20.6	2.3	161
	Avg.	341	20.4	2.5	161
U23	1	265	30.3	3.4	322
	2	204	22.5	4.7	272
	3	318	26.6	3.1	292
	Avg.	262	26.5	3.7	295
U24	1	135	22.1	0.5	366
	2	421	33.7	1.6	136
	3	262	28.5	2.6	182
	Avg.	273	28.1	1.6	228
U29	1	143	27.3	0.6	113
	2	158	5.9	0.8	42
	3	186	19.6	1.3	82
	Avg.	162	17.6	0.9	79
U30	1	332	46.7	1.3	296
	2	280	49.3	4.8	160
	3	322	42.3	4.5	141
	Avg.	311	46.1	3.5	199
U31	1	506	23.1	11.2	189
	2	439	36.7	11.1	301
	3	382	29.2	10.7	133
	Avg.	442	29.7	11.0	208
Smokers					
U01	1	118	10.2	1.2	63
	2	178	26.7	0.9	166
	3	459	33.8	63.8	478
	Avg.	252	23.6	22.0	236
U02	1	251	22.0	3.1	107
	2	562	34.0	1.7	237
	3	1109	24.4	4.2	139
	Avg.	641	26.8	3.0	161
U04	1	136	15.4	3.5	152
	2	302	18.0	3.6	182
	3	363	14.9	4.8	65
	Avg.	267	16.1	4.0	133

Table A.11. (cont.)

U06	1	521	33.0	69.7	346
	2	223	23.2	7.1	286
	3	312	25.3	3.6	116
	Avg.	352	27.2	26.8	249
U13	1	602	24.6	3.0	195
	2	723	12.0	5.1	99
	3	420	53.4	5.4	226
	Avg.	582	30.0	4.5	173
U14	1	219	29.4	1.9	122
	2	122	15.9	0.4	128
	3	113	22.2	1.0	101
	Avg.	151	22.5	1.1	117
U15	1	299	43.3	1.2	102
	2	156	23.7	0.6	158
	3	693	17.0	4.0	167
	Avg.	383	28.0	1.9	142
U16	1	276	36.6	2.3	251
	2	647	66.4	7.3	247
	3	655	35.6	7.0	246
	Avg.	526	46.2	5.5	248
U18	1	936	8.6	6.6	178
	2	283	43.5	4.1	382
	3	361	21.7	3.8	186
	Avg.	527	24.6	4.8	249
U19	1	816	64.8	82.9	591
	2	585	26.0	7.7	212
	3	414	31.3	4.8	190
	Avg.	605	40.7	31.8	331
U21	1	641	17.7	6.3	155
	2	928	49.0	133.3	1121
	3	418	23.1	4.2	144
	Avg.	662	29.9	47.9	473
U25	1	398	46.3	10.1	123
	2	510	20.8	5.0	241
	3	376	24.7	3.8	174
	Avg.	428	30.6	6.3	179

Table A.11. (cont.)

U27	1	193	45.1	4.1	155
	2	271	15.7	0.8	69
	3	82	19.3	2.6	113
	Avg.	182	26.7	2.5	112
U28	1	26	24.0	1.5	862
	2	0	28.5	0	606
	3	27	26.2	0.9	589
	Avg.	18	26.2	0.8	686
U32	1	287	28.9	4.7	143
	2	272	38.5	3.8	187
	3	292	33.2	4.1	157
	Avg.	284	33.5	4.2	162
U33	1	717	19.3	4.7	301
	2	116	21.0	3.3	406
	3	232	23.1	3.9	372
	Avg.	355	21.1	4.0	360
U34	1	329	27.1	4.3	260
	2	174	15.5	7.1	163
	3	374	20.6	5.2	184
	Avg.	292	21.1	5.5	202
U35	1	294	17.1	9.1	152
	2	666	3.5	1.9	151
	3	168	28.0	3.1	239
	Avg.	376	16.2	4.7	181
U38	1	284	9.8	5.0	192
	2	664	21.5	3.8	358
	3	267	19.7	3.6	212
	Avg.	405	17.0	4.1	254

APPENDIX B. Individual Data (rural subjects)

Table B.1. Descriptive characteristics of the subjects.

Subject	Age (y)[1]	Type of Smoking	PA Status
Non-smokers			
R020	30	--	L
R030	31	--	S
R050	42	--	S
R060	35	--	S
R080	23	--	S
R090	46	--	L
R100	33	--	S
R110	34	--	L
R140	34	--	L
R160	29	--	S
R220	24	--	S
R230	23	--	S
R240	34	--	M
R250	34	--	L
R270	31	--	S
Smokers			
R040	30	SC	S
R070	27	SC	L
R130	35	SC	S
R150	32	SC	L
R190	25	SC	L

1: The age was reported in Arabic Calendar, year = 354 days

Table B.2. Height, weight and Body Mass Index (BMI)

Subject	Height (cm)	Weight (kg)	BMI (Kg/m²)
Non-smokers			
R020	171	74.5	25.5
R030	170	85.2	29.5
R050	165	87.4	32.1
R060	163	60.5	23.2
R080	170	75.0	26.0
R090	180	116.0	35.8
R100	163	61.0	23.0
R110	185	122.0	35.7
R140	167	103.0	36.9
R160	178	77.5	24.5
R220	164	55.0	20.5
R230	169	81.0	28.4
R240	183	84.0	25.1
R250	175	87.0	28.4
R270	167	107.0	38.4
Smokers			
R040	170	109.0	37.7
R070	178	66.5	21.0
R130	177	79.0	25.2
R150	160	59.0	23.1
R190	170	72.0	24.9

Table B.3. Hemoglobin and hematocrit

Subject	Hemoglobin (g/L)	Hematocrit (%)
Non-smokers		
R020	166	51
R030	154	48
R050	152	44
R060	156	46
R080	162	50
R090	183	54
R100	163	50
R110	163	49
R140	161	50
R160	144	44
R220	164	49
R230	154	47
R240	165	50
R250	163	49
R270	161	49
Smokers		
R040	200	59
R070	162	49
R130	177	54
R150	163	50
R190	179	53

Table B.4. Plasma B-6 vitamers and red blood cell (RBC) PLP concentration

Subject	Plasma PLP (nmol/L)	Plasma 4-PA (nmol/L)	Plasma PL (nmol/L)	Plasma PN (nmol/L)	RBC PLP (nmol/L)
Non-smokers					
R020	74.5	13.3	16.5	7.6	32.4
R030	48.2	15.2	11.9	9.1	28.1
R050	39.6	10.8	14.8	8.8	--
R060	38.5	11.9	10.4	11.6	27.2
R080	36.3	6.2	9.5	9.0	33.1
R090	46.9	28.9	17.9	9.4	22.7
R100	26.2	10.3	11.0	8.3	25.2
R110	35.8	10.3	8.1	7.1	22.6
R140	24.0	8.8	ND	7.3	23.4
R160	60.8	14.5	18.1	14.6	27.1
R220	53.1	12.3	13.2	7.6	34.7
R230	38.1	10.5	6.2	7.8	19.3
R240	30.2	9.8	11.3	7.9	34.0
R250	55.0	8.7	11.5	7.6	21.7
R270	20.1	11.2	9.7	18.7	24.4
Smokers					
R040	19.9	13.0	11.9	10.1	20.2
R070	59.4	25.7	24.0	8.8	28.3
R130	37.7	9.1	ND	8.3	26.2
R150	28.9	12.0	11.3	12.1	24.3
R190	37.1	7.0	7.5	8.3	31.9

Table B.5. Plasma alkaline phosphatase activity and albumin concentration

Subject	Alkaline phosphatase (U/L)	Albumin (g/L)
Non-smokers		
R020	25.0	56
R030	18.2	51
R050	22.6	51
R060	32.7	52
R080	34.0	55
R090	32.5	54
R100	25.9	52
R110	15.0	54
R140	27.5	50
R160	13.8	50
R220	21.5	53
R230	29.0	50
R240	24.3	53
R250	24.0	51
R270	29.8	50
Smokers		
R040	39.8	53
R070	28.1	52
R130	26.5	59
R150	26.8	55
R190	29.2	53

Table B.6. Urinary 4-PA, creatinine and urea nitrogen excretion and 4-PA/creatinine ratio.

Subject	Day #	4-PA (μmol/day)	Creatinine (g/day)	4-PA/ Creatinine ratio	Urea N. (g/day)
Non-smokers					
R020	1	3.3	1.2	2.7	8.1
	2	1.4	0.7	1.9	3.7
	3	2.0	1.0	2.0	5.8
	Avg.	2.2	1.0	2.2	5.9
R030	1	3.1	1.0	3.2	4.8
	2	3.8	1.2	3.2	6.3
	3	4.7	1.3	3.5	7.0
	Avg.	3.9	1.2	3.3	6.0
R050	1	4.4	1.7	2.6	7.7
	2	4.3	1.7	2.6	9.6
	3	5.2	1.7	3.1	4.7
	Avg.	4.6	1.7	2.8	7.3
R060	1	4.7	1.2	4.0	5.6
	2	4.3	1.3	3.3	5.4
	3	4.8	1.3	3.6	7.7
	Avg.	4.6	1.3	3.6	6.2
R080	1	4.1	1.4	2.9	7.4
	2	2.7	1.1	2.4	6.6
	3	2.3	1.3	1.8	5.5
	Avg.	3.0	1.3	2.4	6.5
R090	1	5.6	1.7	3.4	12.2
	2	2.8	0.9	3.2	5.8
	3	7.3	1.6	4.5	8.0
	Avg.	5.2	1.4	3.7	8.7
R100	1	3.3	1.0	3.3	6.1
	2	4.3	1.3	3.3	8.3
	3	4.3	1.3	3.3	9.2
	Avg.	4.0	1.2	3.3	7.9

Table B.6. (cont.)

R110	1	4.8	1.6	3.1	7.0
	2	4.9	1.6	3.1	9.8
	3	5.3	1.7	3.1	10.2
	Avg.	5.0	1.6	3.1	9.0
R140	1	3.4	1.6	2.2	8.6
	2	3.1	1.3	2.4	7.7
	3	3.7	1.6	2.3	10.9
	Avg.	3.4	1.5	2.3	9.1
R160	1	6.4	1.6	4.0	11.0
	2	5.7	1.6	3.6	10.3
	3	5.7	1.8	3.3	11.0
	Avg.	5.9	1.7	3.6	10.8
R220	1	4.4	0.9	4.6	5.9
	2	4.0	1.1	3.8	6.4
	3	2.3	0.7	3.4	3.7
	Avg.	3.6	0.9	3.9	5.3
R230	1	1.9	0.7	2.6	3.9
	2	3.0	1.2	2.6	8.4
	3	3.9	1.4	2.8	8.5
	Avg.	2.9	1.1	2.7	6.9
R240	1	3.4	1.4	2.4	6.3
	2	0.5	1.1	0.5	7.2
	3	2.7	1.2	2.3	6.2
	Avg.	2.2	1.2	1.7	6.6
R250	1	3.6	1.2	3.0	6.9
	2	5.2	1.3	3.8	10.1
	3	5.4	1.6	2.1	8.7
	Avg.	4.7	1.4	3.0	8.6
R270	1	6.6	2.2	3.0	13.6
	2	6.4	2.1	3.0	16.0
	3	5.4	2.1	2.6	14.9
	Avg.	6.1	2.1	2.9	14.8

Table B.6. (cont.)

Smokers					
R040	1	4.3	1.7	2.6	14.1
	2	5.1	1.9	2.8	11.5
	3	3.9	1.5	2.6	9.6
	Avg.	4.4	1.7	2.7	11.7
R070	1	13.3	1.2	10.7	6.3
	2	13.8	1.4	9.6	7.2
	3	12.0	1.3	9.3	6.3
	Avg.	13.0	1.3	9.9	6.6
R130	1	4.1	1.7	2.5	8.0
	2	3.9	1.7	2.4	7.9
	3	3.6	1.3	2.7	6.9
	Avg.	3.9	1.6	2.5	7.6
R150	1	2.3	0.7	3.2	3.8
	2	3.1	1.1	2.8	6.0
	3	3.7	1.3	2.7	5.9
	Avg.	3.0	1.0	2.9	5.2
R190	1	3.1	1.2	2.6	6.2
	2	2.8	1.3	2.1	7.2
	3	2.9	1.2	2.4	6.6
	Avg.	2.9	1.2	2.4	6.7

Table B.7. Intake of vitamin B-6, dietary protein, B-6:protein ratio, B-6 and protein from animal source

Subject	Day #	Vitamin B-6 (mg)	Protein (g)	B-6:protein ratio(mg/g)	B-6 from animal source (%)	Protein from animal source(%)
Non-smokers						
R020	1	3.84	156	0.025	61.0	81.0
	2	3.61	182	0.020	78.0	85.0
	3	3.74	166	0.023	67.0	83.0
	Avg.	3.73	168	0.023	68.7	83.0
R030	1	1.24	76	0.016	51.0	68.0
	2	2.53	106	0.024	65.0	80.0
	3	2.47	99	0.025	67.0	78.0
	Avg.	2.08	94	0.022	61.0	75.3
R050	1	1.95	103	0.019	60.0	66.0
	2	2.57	62	0.041	22.0	44.0
	3	2.12	97	0.022	57.0	68.0
	Avg.	2.21	87	0.027	46.3	59.3
R060	1	2.10	75	0.028	25.0	48.0
	2	2.07	81	0.026	54.0	53.0
	3	1.97	94	0.021	61.0	61.0
	Avg.	2.05	83	0.025	46.7	54.0
R080	1	1.52	85	0.018	60.0	70.0
	2	0.98	73	0.013	26.0	60.0
	3	1.43	90	0.016	58.0	64.0
	Avg.	1.31	83	0.016	48.0	64.7
R090	1	2.41	106	0.023	60.0	71.0
	2	2.45	157	0.016	53.0	74.0
	3	2.39	121	0.020	57.0	65.0
	Avg.	2.42	128	0.020	56.7	70.0
R100	1	1.92	52	0.036	21.0	40.0
	2	1.79	88	0.020	22.3	46.7
	3	1.31	67	0.019	19.8	41.2
	Avg.	1.67	69	0.025	21.0	42.6

Table B.7. (cont.)

R110	1	2.29	102	0.023	53.0	68.0
	2	1.76	107	0.016	61.0	77.0
	3	2.21	93	0.024	67.0	73.0
	Avg.	2.09	101	0.021	60.3	72.7
R140	1	1.31	98	0.013	53.0	68.0
	2	1.45	98	0.015	62.0	70.0
	3	2.06	88	0.023	48.0	63.0
	Avg.	1.61	95	0.017	54.3	67.0
R160	1	3.75	155	0.024	68.0	80.0
	2	3.56	179	0.020	73.0	86.0
	3	3.84	172	0.022	69.0	84.0
	Avg.	3.72	169	0.022	70.0	83.3
R220	1	2.31	85	0.027	64.0	77.0
	2	2.11	76	0.028	60.0	74.0
	3	1.98	95	0.021	68.0	83.0
	Avg.	2.13	85	0.025	64.0	78.0
R230	1	1.93	103	0.019	53.0	67.0
	2	2.54	114	0.022	47.0	53.0
	3	2.07	99	0.021	55.0	68.0
	Avg.	2.18	105	0.021	51.7	62.7
R240	1	1.51	102	0.015	49.0	65.0
	2	1.63	92	0.018	53.0	61.0
	3	1.71	87	0.020	46.0	57.0
	Avg.	1.62	94	0.018	49.3	61.0
R250	1	1.31	78	0.017	64.0	73.0
	2	2.58	107	0.024	68.0	85.0
	3	2.51	101	0.025	66.0	83.0
	Avg.	2.13	95	0.022	66.0	80.3
R270	1	2.43	142	0.017	77.0	75.0
	2	1.73	121	0.014	79.0	63.0
	3	1.26	98	0.013	76.0	74.0
	Avg.	1.81	120	0.014	77.3	70.7

Table B.7. (cont.)

Smokers						
R040	1	3.61	257	0.014	81.0	86.0
	2	2.33	98	0.023	44.0	50.0
	3	1.87	96	0.019	46.0	53.0
	Avg.	2.60	150	0.019	57.0	63.0
R070	1	2.77	95	0.029	64.0	74.0
	2	2.30	97	0.024	55.0	72.0
	3	3.11	102	0.031	69.0	82.0
	Avg.	2.73	98	0.028	62.7	76.0
R130	1	2.13	96	0.022	61.0	75.0
	2	1.87	97	0.019	58.0	73.0
	3	2.08	98	0.021	67.0	80.0
	Avg.	2.03	97	0.021	62.0	76.0
R150	1	1.68	97	0.017	56.0	69.0
	2	1.66	78	0.021	60.0	72.0
	3	1.23	87	0.014	53.0	68.0
	Avg.	1.52	87	0.017	56.3	69.7
R190	1	1.03	61	0.017	47.0	61.0
	2	1.34	83	0.016	53.0	63.0
	3	1.92	78	0.024	41.0	58.0
	Avg.	1.43	74	0.019	47.0	60.7

Table B.8. Intake of soluble dietary fiber (SDF), insoluble dietary fiber (IDF), riboflavin and zinc.

Subject	Day #	SDF (g)	IDF (g)	Riboflavin (mg)	Zn (mg
Non-smokers					
R020	1	1.7	2.5	1.85	10.8
	2	4.3	14.1	2.33	19.2
	3	3.9	7.6	1.91	11.2
	Avg.	3.3	8.1	2.03	13.7
R030	1	3.3	8.8	1.56	7.5
	2	3.4	7.2	1.46	7.3
	3	3.7	8.4	1.61	7.5
	Avg.	3.5	8.1	1.54	7.4
R050	1	4.9	18.4	1.68	13.1
	2	7.6	22.3	1.36	7.5
	3	6.5	19.8	1.44	10.2
	Avg.	6.3	20.2	1.49	10.3
R060	1	8.9	28.5	1.90	9.9
	2	10.1	28.7	2.13	10.1
	3	9.8	29.1	1.83	8.8
	Avg.	9.6	28.8	1.95	9.6
R080	1	3.6	6.7	1.99	13.0
	2	3.6	7.5	1.51	11.8
	3	4.1	9.5	1.83	9.8
	Avg.	3.8	7.9	1.78	11.5
R090	1	5.1	12.4	2.16	13.7
	2	4.5	13.5	2.41	10.2
	3	5.1	14.7	2.37	11.5
	Avg.	4.9	13.5	2.31	11.8
R100	1	2.5	7.0	0.94	5.1
	2	2.6	6.7	1.28	5.3
	3	2.7	6.7	1.13	4.9
	Avg.	2.6	6.8	1.17	5.1
R110	1	4.8	10.8	2.06	14.1
	2	5.1	11.8	2.31	12.1
	3	4.7	13.7	1.96	10.9
	Avg.	4.9	12.1	1.12	12.4

Table B.8. (cont.)

R140	1	4.1	11.4	1.73	12.1
	2	3.7	11.8	2.13	9.8
	3	4.7	12.3	2.21	10.5
	Avg.	4.2	11.8	2.02	10.8
R160	1	1.7	3.5	1.86	10.7
	2	4.2	10.1	1.93	11.3
	3	4.9	11.7	2.12	10.4
	Avg.	3.6	8.4	1.97	10.8
R220	1	6.9	13.2	1.70	7.9
	2	8.1	11.8	1.98	11.1
	3	7.9	12.6	1.75	7.8
	Avg.	7.6	12.5	1.81	8.9
R230	1	4.9	9.1	1.66	11.1
	2	8.2	11.3	1.34	6.6
	3	5.2	10.6	1.41	9.7
	Avg.	6.1	10.3	1.47	9.1
R240	1	4.7	8.7	1.53	9.7
	2	7.1	13.7	1.49	7.6
	3	6.2	14.1	1.26	8.7
	Avg.	6.0	12.2	1.43	8.7
R250	1	3.9	8.9	1.65	8.6
	2	4.6	9.2	1.64	7.7
	3	3.8	8.6	1.91	6.2
	Avg.	4.1	8.9	1.73	7.5
R270	1	3.1	10.3	2.09	11.2
	2	3.3	8.9	4.17	13.2
	3	2.9	11.2	1.81	10.1
	Avg.	3.1	10.1	2.69	11.5
Smokers					
R040	1	5.9	11.5	2.25	14.4
	2	5.1	12.4	2.34	8.9
	3	4.9	11.3	3.61	7.8
	Avg.	5.3	11.7	2.73	10.4

Table B.8. (cont.)

R070	1	3.8	6.2	0.91	5.8
	2	1.6	3.2	1.67	10.6
	3	3.2	6.7	2.18	6.5
	Avg.	2.9	5.4	1.59	7.6
R130	1	3.9	6.3	1.10	6.1
	2	2.4	5.9	1.27	11.7
	3	3.1	7.2	2.31	7.8
	Avg.	3.1	6.5	1.56	8.5
R150	1	1.2	3.4	1.84	10.7
	2	1.1	3.0	1.65	9.9
	3	2.3	4.2	1.79	10.3
	Avg.	1.5	3.5	1.76	10.3
R190	1	2.2	5.2	1.23	7.8
	2	3.2	7.8	1.37	9.1
	3	1.6	4.2	3.59	10.2
	Avg.	2.3	5.7	2.06	9.0

Table B.9. Intake of calories, carbohydrates, fat, saturated fat (S-fat), and unsaturated fat (U-fat)

Subject	Day #	Calories (K.cal)	Carbohyd-rates (g)	Fat (g)	S-Fat (g)	U-Fat (g)
Non-smokers						
R020	1	2745	287	113	29	76
	2	2734	307	88	22	55
	3	2733	308	93	22	62
	Avg.	2737	301	98	24	64
R030	1	2261	266	104	47	47
	2	2315	303	78	23	48
	3	2372	272	98	41	48
	Avg.	2316	280	93	37	48
R050	1	2213	349	54	15	34
	2	2196	416	44	9	30
	3	2205	348	49	12	32
	Avg.	2205	371	49	12	32
R060	1	2066	379	38	12	22
	2	2117	378	36	12	19
	3	2049	339	37	13	21
	Avg.	2077	365	37	12	20
R080	1	2299	299	85	31	47
	2	2440	322	95	36	52
	3	2318	302	84	33	45
	Avg.	2352	308	88	33	48
R090	1	3288	369	151	66	74
	2	3391	423	117	48	61
	3	3127	443	96	23	56
	Avg.	3269	412	121	46	64
R100	1	2021	324	62	25	32
	2	1874	281	49	21	23
	3	2009	304	60	22	35
	Avg.	1968	303	57	23	30

Table B.9. (cont.)

R110	1	3356	556	79	25	48
	2	3395	599	66	17	43
	3	3316	545	91	32	52
	Avg.	3356	567	79	25	48
R140	1	2637	319	109	24	77
	2	2650	361	91	21	62
	3	2662	410	74	18	48
	Avg.	2650	363	91	21	62
R160	1	2378	260	82	25	46
	2	2371	255	72	24	38
	3	2383	233	90	26	55
	Avg.	2377	249	81	25	46
R210	1	2071	272	86	34	41
	2	2101	280	86	31	44
	3	2172	287	84	25	39
	Avg.	2115	280	85	30	41
R220	1	1929	275	65	17	41
	2	1913	301	52	20	27
	3	1963	279	53	17	30
	Avg.	1935	285	57	18	33
R230	1	2512	314	92	32	52
	2	2493	319	84	36	38
	3	2533	333	89	34	45
	Avg.	2513	322	88	34	45
R240	1	3068	326	154	47	80
	2	3147	381	89	25	50
	3	3080	504	82	22	47
	Avg.	3098	404	108	31	59
R250	1	2885	351	130	49	58
	2	2790	343	122	50	60
	3	2836	322	125	46	56
	Avg.	2837	339	126	48	58

Table B.9. (cont.)

R270	1	2499	251	103	37	59
	2	2781	360	94	24	60
	3	2668	363	90	26	56
	Avg.	2649	325	96	29	58
Smokers						
R040	1	2643	163	111	33	71
	2	2810	369	107	23	73
	3	2733	363	99	22	66
	Avg.	2729	298	106	26	70
R070	1	2511	342	90	28	56
	2	2467	337	82	32	44
	3	2517	330	87	35	47
	Avg.	2498	336	86	32	49
R130	1	2131	306	64	20	38
	2	2230	263	92	32	50
	3	2163	255	84	29	46
	Avg.	2175	275	80	27	45
R150	1	1986	263	59	18	34
	2	2022	335	47	13	31
	3	1979	312	43	11	28
	Avg.	1996	303	50	14	31
R190	1	2737	417	91	28	52
	2	2723	440	70	24	15
	3	2718	425	78	24	42
	Avg.	2726	427	80	25	36

Table B.10. Intake of vitamin C, vitamin B1, vitamin D, calcium, and iron

Subject	Day #	Vitamin C (mg)	Vitamin B1 (mg)	Vitamin D (IU)	Calcium (mg)	Iron (mg)
Non-smokers						
R020	1	281	1.9	48	1351	48.4
	2	7	1.2	50	614	18.3
	3	92	1.3	47	741	16.1
	Avg.	127	1.5	48	902	27.6
R030	1	271	1.5	16	632	12.4
	2	420	1.2	25	621	12.2
	3	303	1.4	29	628	11.8
	Avg.	331	1.4	23	627	12.1
R050	1	47	4.8	36	350	12.3
	2	84	1.7	5	364	12.4
	3	57	1.9	26	381	12.1
	Avg.	63	2.8	22	365	12.3
R060	1	411	2.0	13	946	11.3
	2	73	1.7	28	932	11.5
	3	92	1.9	20	942	11.1
	Avg.	192	1.9	20	940	11.3
R080	1	12	1.6	6	475	10.8
	2	8	1.4	34	355	9.9
	3	11	1.5	46	414	10.2
	Avg.	10	1.5	29	415	10.3
R090	1	331	1.4	46	812	11.7
	2	228	1.6	19	941	10.3
	3	247	1.7	25	714	10.8
	Avg.	269	1.6	30	822	10.9
R100	1	282	0.9	9	303	8.8
	2	123	0.8	25	669	7.2
	3	190	0.9	32	413	8.1
	Avg.	198	0.9	22	462	8.0
R110	1	331	1.5	38	377	12.3
	2	236	1.7	37	1032	11.3
	3	287	1.7	36	615	11.9
	Avg.	285	1.6	37	675	11.8

Table B.10. (cont.)

R140	1	22	2.3	0	431	13.1
	2	33	1.4	47	1702	8.6
	3	27	1.8	27	620	10.2
	Avg.	27	1.8	25	918	10.6
R160	1	109	1.8	23	1114	12.4
	2	191	1.7	28	517	13.2
	3	172	1.6	26	612	11.8
	Avg.	157	1.7	26	748	12.5
R210	1	8	0.6	15	203	9.1
	2	73	0.9	25	589	10.6
	3	84	0.8	19	392	9.3
	Avg.	55	0.8	20	395	9.7
R220	1	85	1.0	41	297	12.6
	2	243	2.0	20	454	13.6
	3	163	1.4	24	517	11.9
	Avg.	164	1.5	28	423	12.7
R230	1	54	1.5	55	358	9.4
	2	50	2.9	29	1461	11.3
	3	86	1.8	32	961	10.2
	Avg.	63	2.1	39	927	10.3
R240	1	198	1.1	7	1168	13.4
	2	120	1.8	55	1400	16.3
	3	146	1.4	41	1103	12.7
	Avg.	155	1.4	34	1224	14.1
R250	1	60	1.6	58	929	17.0
	2	26	1.5	19	339	12.3
	3	47	1.5	42	412	11.8
	Avg.	44	1.5	40	560	13.7
R270	1	13	1.6	44	969	11.8
	2	7	3.5	18	625	17.3
	3	9	1.7	32	814	11.2
	Avg.	10	2.3	31	803	13.4

Table B.10. (cont.)

Smokers						
R040	1	40	1.9	272	990	10.2
	2	174	2.6	13	515	15.0
	3	122	2.1	8	714	9.8
	Avg.	112	2.2	98	740	11.7
R070	1	253	1.1	29	292	7.1
	2	69	1.0	23	752	10.1
	3	93	1.0	26	513	8.3
	Avg.	138	1.0	26	519	8.5
R130	1	182	1.2	179	746	7.1
	2	59	1.7	24	1042	9.6
	3	112	1.5	29	931	8.7
	Avg.	118	1.5	77	906	8.5
R150	1	76	1.4	29	786	12.0
	2	207	1.2	20	696	10.0
	3	124	1.2	24	715	10.8
	Avg.	136	1.3	24	732	10.9
R190	1	113	1.7	34	639	18.6
	2	51	0.5	22	1110	9.9
	3	84	1.1	29	931	10.2
	Avg.	83	1.1	28	893	12.9

Table B.11. Intake of cholesterol, vitamin B3, vitamin B12, and folate

Subject	Day #	Cholesterol (mg)	Vitamin B3 (mg)	Vitamin B12 (µg)	Folate (µg)
Non-smokers					
R020	1	373	59.1	2.5	435
	2	822	56.6	8.4	115
	3	481	57.3	2.2	147
	Avg.	559	57.7	4.4	232
R030	1	303	22.4	3.2	282
	2	633	34.6	2.8	317
	3	511	27.2	2.4	299
	Avg.	482	28.1	2.8	299
R050	1	275	36.6	3.4	132
	2	431	21.4	1.4	299
	3	313	28.7	2.3	173
	Avg.	340	28.9	2.4	201
R060	1	90	25.3	1.9	386
	2	438	40.5	5.1	165
	3	171	31.4	2.8	187
	Avg.	233	32.4	3.3	246
R080	1	270	23.1	6.5	52
	2	227	23.3	4.5	88
	3	318	29.2	3.2	72
	Avg.	272	25.2	4.7	71
R090	1	511	47.2	3.6	162
	2	583	38.1	4.2	112
	3	604	35.6	3.8	149
	Avg.	566	40.3	3.9	141
R100	1	201	17.6	0.6	134
	2	244	31.6	2.1	111
	3	268	24.5	1.9	122
	Avg.	238	24.6	1.5	122
R110	1	413	55.4	1.2	257
	2	287	47.6	2.7	279
	3	334	31.7	1.8	261
	Avg.	345	44.9	1.9	266

Table B.11. (cont.)

R140	1	220	29.1	6.4	225
	2	313	43.8	4.0	136
	3	271	35.2	3.8	189
	Avg.	268	36.0	4.7	183
R160	1	591	29.1	5.9	243
	2	619	38.1	24.9	242
	3	737	26.7	4.2	237
	Avg.	649	31.3	11.7	240
R210	1	246	10.4	3.7	72
	2	361	18.3	4.0	174
	3	398	14.5	2.8	138
	Avg.	335	14.4	3.5	128
R220	1	217	21.1	0.7	181
	2	119	16.4	2.0	219
	3	192	19.8	1.9	207
	Avg.	176	19.1	1.5	202
R230	1	450	67.2	1.9	106
	2	266	38.5	3.5	172
	3	342	27.8	2.7	133
	Avg.	353	44.5	2.7	137
R240	1	619	15.3	5.8	134
	2	641	56.3	5.2	242
	3	893	18.7	3.6	167
	Avg.	718	30.1	4.9	181
R250	1	534	72.2	3.7	105
	2	342	19.4	4.3	277
	3	412	23.7	2.8	215
	Avg.	429	38.4	3.6	199
R270	1	534	43.6	3.1	317
	2	713	49.3	29.8	481
	3	391	46.5	2.8	328
	Avg.	546	46.5	11.9	375

Table B.11. (cont.)

Smokers					
R040	1	270	61.3	8.0	175
	2	699	45.7	4.9	257
	3	463	32.4	2.2	215
	Avg.	477	46.5	5.0	216
R070	1	271	36.6	1.0	107
	2	271	26.8	4.1	165
	3	283	31.2	1.8	188
	Avg.	275	31.5	2.3	153
R130	1	142	28.5	3.1	208
	2	388	21.1	2.6	168
	3	411	19.4	2.9	190
	Avg.	314	23.0	2.9	189
R150	1	649	38.0	4.5	276
	2	161	31.8	3.2	224
	3	278	24.2	2.9	256
	Avg.	363	31.3	3.5	252
R190	1	1062	47.8	3.5	293
	2	246	29.1	0.6	110
	3	286	37.0	1.9	173
	Avg.	531	38.0	2.0	192

APPENDIX C. Forms and Instructions

Figure C.1 Translation of the recruiting flyer

We Need Volunteers

Investigators from the Department of Nutrition and Food Management at Oregon State University – U.S.A. & Department of Food Sciences and Nutrition at King Saud University need healthy Saudi adult male volunteers to participate in a study titled "Evaluation of Vitamin B-6 Status of Saudi Adult Males in the Riyadh Region – Saudi Arabia."

Benefits:

- **Free evaluation of your dietary intake.**
- **Confidential report of blood analysis results.**
- **Get answers to nutritional questions you may have from American professor in the field of nutrition "Dr. James Leklem."**

If you are interested call Abdullah Al-Assaf at

Home:	**450-0759**
Mobile:	**05-315-5447**
Lab:	**467-8249**

Figure C.2. Translation of the questionnaire form

Name: _____

Phone number(s) where we can call you: (Day time) _____

(Night time) _____

Age: _____ Weight: _____ (kg) Height: _____ (cm)

(1) How do you describe your physical activity status ?

(sedentary) (light) (moderate) (hard)

(2) Do you exercise? (Yes) (No)

If you answered yes, how many times per week?: _____

How many hours per time?: _____

How do you describe your intensity of exercise? (Heavy) (Moderate) (Light)

(3) Has your weight changed during the last two months? (Yes) (No)

If you answer yes, please specify_____

(4) Are you in any type of diet?

If you answer yes, please specify _____

(5) Do you presently smoke? (Yes) (No)

What type?: cigarette, pipe, cigar, others _____

How many cigarettes, cigar, others per day? _____

How long have you smoked?: _____

Does anyone in your household smoke? (Yes) (No)

(6) Do you have any chronic diseases? (Yes) (No)

If you answered yes, please list them: _____

If you use any kind of medicines, please list them:_____

How do you describe your health status? _____

(7) Do you use any kind of dietary supplements? (Yes) (No)

If you answered yes, please list them: _____

How long have you used each? _____

When is the last time you took a dietary supplement? _____

Which one(s) was it? _____

Figure C.3. Translation of the instructions for urine collection and food record.

Urine:

Start the collection of three consecutive days at specified time; i.e., 7:00 a.m., discard the first urine excretion on the first day. Collect all urine after 7:00 a.m., including the 7:00 a.m. specimen of the following morning in the bottle of the first day. Collect all urine after 7:00 a.m. of the second day and the 7:00 a.m. specimen of the third day morning in the bottle of the second day. Collect all urine after 7:00 a.m. of the third day and the 7:00 a.m. specimen of the fourth day morning in the bottle of the third day. Store all bottles in a cool, dark, dry place. Bring all bottles with you to the laboratory in the morning of the fourth day – blood drawing day.

Food:

Record all foods and drinks you consume on each day of those days in the provided tables. Do not just record name of dishes, instead write in the description and preparation section all ingredients of the dish and their amounts; i.e., oil, sauces, spices. If food was obtained from a restaurant, write the name of the restaurant in that section. To determine the amount, use these guidelines:

- Beverages: use the provided cup.
- Fruits and vegetables: record the size as small, medium, large. For slices, i.e., watermelon, use a ruler to record length and thickness. For legumes and leafy vegetables, use the provided cup.
- Meat: Use the provided chart.
- Fats; i.e., oil, butter: Use the provided spoon.
- Packed foods: Use the amount written on the pack.

Remember:

Bring your food records and urine bottles to the nutrition laboratory on the blood drawing day. You must come in a fasting state and you must drink one cup of water before you leave your home. After blood drawing, we will provide you a delicious healthy breakfast! **If you have any questions during these three days, please do not hesitate to call Abdullah at any time at his mobile phone: 05-315-5447.**

Figure C.4. Food record form

Name: Code #:

Date: Day of week: Day #:

Time	Food Item	Amount	Description and preparation

Fig. C.5. Chart of meat amount determination

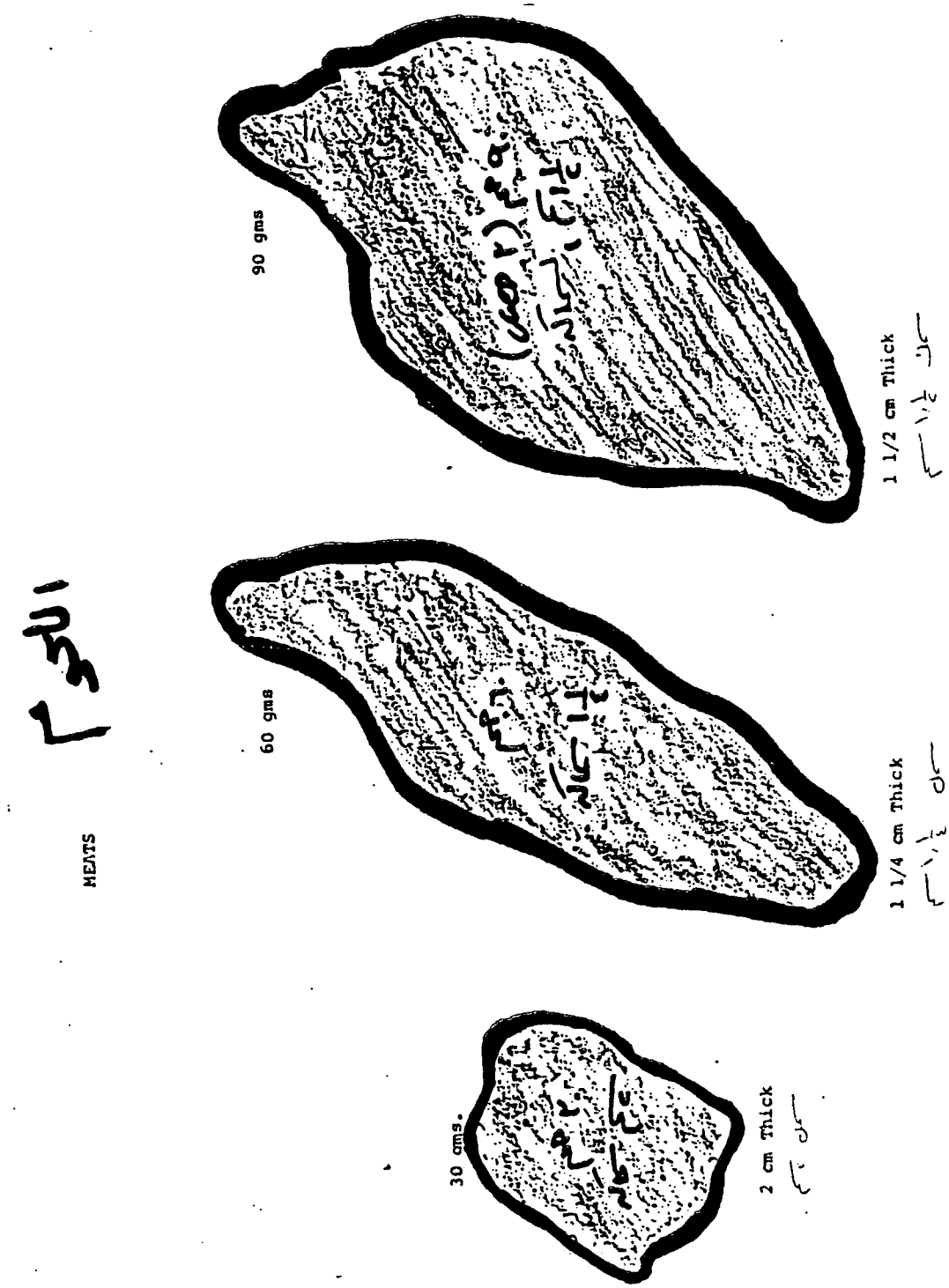

Figure C.6. Informed consent form.

A. Title:
Evaluation of vitamin B-6 status of Saudi adult males in the Riyadh region-Saudi Arabia.

B. Investigators:
Dr. James E. Leklem and Abdullah H. Al-Asaf. Department of Nutrition and Food Management, Oregon State University, Corvallis, Oregon, USA.

C. Purpose of the research project:
To evaluate vitamin B-6 status of Saudi adult male subjects by determining the dietary intake of vitamin B-6 and protein and measuring vitamin B-6 vitamers in plasma as well as in urine.

D. Procedures:
I have received an oral explanation of this research and I volunteer to answer questions in the questionnaire which will determine my eligibility to participate in the research. If I participate in this research, I understand that I will do the following:

- I will not take any dietary supplements during the period of this study.
- If I need to take any medications during this study, I will inform the investigators.
- I will record the foods I consume for three days (2 weekdays and 1 weekend day).
- I will collect my complete 24-hour urine excretion into a supplied container each day of the three-day food records and bring it to laboratory at King Saud University with my food record of that day.
- On the morning of the fourth day, I will come to the laboratory in a fasting state for blood drawing. A 20 ml of blood (equivalent to about two tablespoons) will be drawn from the forearm vein by a registered nurse from King Saud University hospital.
- I will not perform any strenuous exercise during the research period. I will maintain a uniform daily schedule of physical activity.

E. Benefits and Risks:
The benefits I will receive from participation in this research will include an evaluation of my dietary intake for various nutrients and the results of vitamin B-6 status as well as hematocrit and hemoglobin values. I will also have an opportunity to discuss my results with the investigators. There will be no risks associated with participation in this research except a minor discomfort from blood drawing when the needle enters the vein, and slight bruising. There are no risks associated with urine collection apart from the obvious disadvantages with carrying around a urine collection bottle.

F. Confidentiality:
I understand that any information obtained from me and my results will be kept confidential and no one will have an access to them except the investigators. A code number will be used instead of my name during the research. My name will not be used in any report or publications that come from this research. Blood drawing and discussion of instructions or results of this research will be done individually.

G. Voluntary participation statement:
My participation in this research is completely voluntary, and I have the right to refuse or discontinue my participation at any time of this research with no penalty.

H. Questions:
If I have any questions regarding this research, I will contact Abdullah Al-Asaf at (01) 450-0759 or (05) 423-7930 who will be pleased to answer them. If I have any questions about my rights as a research subject, I should contact the Oregon State University Institutional Review Board Coordinator by email at IRB@orst.edu.

Understanding and compliance:
My signature below approves that I have read and understand the procedures above and give my informed and voluntary consent to participate in this research. I will keep a signed copy of this consent form.

Signature of subject Date

Name of subject

Subject's present address

Subject's phone number

Signature of investigator Date