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Title: The Effect of Wheat Bran on the Bioavailability of Vitamin B_6 in Humans

Abstract approved: Lorraine T. Miller
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The effect of wheat bran on the bioavailability of vitamin B_6 was determined in 10 men aged 20 to 34 years. The study consisted of a 4-day adjustment period followed by two 18-day and one 19-day experimental period, in that order. The subjects were randomly assigned to two groups. All subjects consumed a controlled diet during the adjustment period and the experimental diet with or without wheat bran during the remaining 55 days of the study. Group 1 consumed 15 g of wheat bran/day in addition to the experimental diet during the first and third periods. Group 2 ate the additional 15 g of wheat bran/day only during the second period. The vitamin B_6 intake was 1.59 mg/day during the adjustment period, 1.69 mg/day during the bran periods, and 1.66 mg/day during the non-bran periods. Dietary neutral detergent fiber was 3.63, 13.7, and 6.6 g/day during the adjustment, bran, and non-bran periods, respectively. Blood was drawn from fasting subjects every third morning for the determination of plasma total vitamin B_6 and pyridoxal phosphate (PLP). Complete urine and fecal collections were made. Urinary vitamin B_6 and 4-pyridoxic acid (4-PA) were determined every 3 days. Three-day fecal composites were analyzed for vitamin B_6. Although there were no statistically significant differences between the bran and non-bran treatments for any of the parameters studied, the data show consistent trends. Bran consistently increased fecal vitamin B_6 in five men and depressed urinary vitamin B_6 and 4-PA in six men. For both groups, while the mean urinary 4-PA decreased, the mean fecal vitamin B_6 increased with bran supplementation. Bran also lowered plasma vitamin B_6 in eight subjects and plasma PLP in five subjects. There were statistically significant differences between the urinary and plasma vitamin B_6 and plasma PLP values for the two groups. There was a

significant negative correlation between urinary 4-PA and fecal vitamin B_6. The results of this study suggest that 15 g of wheat bran does not adversely affect vitamin B_6 status when intake of the vitamin is adequate.

The Effect of Wheat Bran on the Bioavailability of Vitamin B_6 in Humans

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for my family

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INTRODUCTION

During the past few years, dietary fiber has captured scientific and public attention as a possible means to maintain and improve health. Epidemiologic evidence, which admittedly has some limitations, implicates a low intake of fiber as an etiologic factor in numerous deseases. The incidence of certain disorders, especially those of the colonsuch as constipation, diverticular disease, irritable colon, appendicitis, hemorrhoids, ulcerative colitis, and cancer of the colon, is much lower in rural Africa, and in the rural population of some Asian and Pacific countries than in westem, developed countires. People in westem countries consume low amounts of unrefined or lightly refined fiber-rich foods in comparison to people in developing countries. The average crude fiber intake of adults in developed countries has been estimated to be from 3 to 12 g/day. In contrast, the average crude fiber intake in developing African countries is 12 to 25 g/day (Trowell, 1977).

Experimental evidence has shown that fiber increases stool weight, and accelerates intestinal transit time in subjects with initially slow transit times (Kelsay, 1978). However, there may be some deleterious effects when large quantities of fiber are consumed. The fecal excretion of various cations increases when the intake of wheat bran is increased (Cummings et al., 1976; Eastwood and Mitchell, 1976). In vitro experiments reveal that bran acts as a poly-functional cation exchanger which increases excretion and decreases absorption of selected nutrients (Reinhold et al., 1975; Ismail-Beiji et al., 1977). Iranian villagers often suffer from hypogonadal dwarfism caused by zinc and iron deficiencies, even though their diet is adequate in these nutrients. These deficiences appear to be related to the villagers' consumption of large quantities of high-fiber bread as a dietary staple (Ismail-Beiji et al., 1977).

The bioavailability of other nutrients may also be affected by a high intake of fiber. A recent experiment compared the bioavailability of vitamin B_6 in

subjects consuming whole wheat bread, white bread to which crystolline vitamin B_6 was added, and white bread plus an oral vitamin B_6 supplement. The subjects' fecal vitamin B_6 was significantly increased with whole wheat bread. Urinary excretion of 4-pyridoxic acid (Perera, 1977) and plasma levels of vitamin B_6 and pyridoxal phosphate (Peffers, 1977) were lower during consumption of whole wheat bread than either of the two white breads.

Wheat bran and wheat bran products are currently popular sources of dietary fiber. The above-mentioned studies suggest that an increase in the intake of wheat bran may adversely affect the bioavailability of vitamin B_6. The present experiment was conducted to explore more fully the effects of wheat bran on the bioavailability of vitamin B_6. While the dietary consumption of vitamin B_6 and other nutrients was held constant, 10 healthy male subjects ate in addition either 0 or 15 g of wheat bran. Throughout the 59-day experiment fecal vitamin B_6 was measured. The absorption of vitamin B_6, as reflected by plasma total vitamin B_6 and pyridoxal phosphate levels and the urinary excretion of the vitamin and its metabolite 4-pyridoxic acid, was determined. Values for these parameters while the subjects received the constant diet with and without wheat bran supplementation were compared. The results of this experiment may provide further insight into the various factors that affect the bioavailability of vitamin B_6.

REVIEW OF LITERATURE

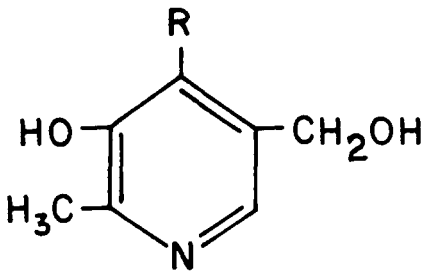
Vitamin B_6

During the 1930's extensive research by various laboratories on the B-complex vitamins lead to the discovery of vitamin B_6. In 1934, Gyorgy identified a "rat acrodynia preventative factor," distinct from riboflavin, that he termed vitamin B_6 (Gyorgy, 1971). As reviewed by Brin (1978), within the decade that followed, vitamin B_6 was firmly established as an essential nutrient for animals and was isolated in pure crystalline form. When its pyridine ring structure was elucidated in 1939, vitamin B_6 was given the more descriptive name, pyridoxine (Gyorgy, 1971). After the discovery of vitamin B_6, almost twenty years elapsed before the human requirement for the vitamin was confirmed by the work of Synderman and coworkers (1952) with vitamin B_6-deficient infants.

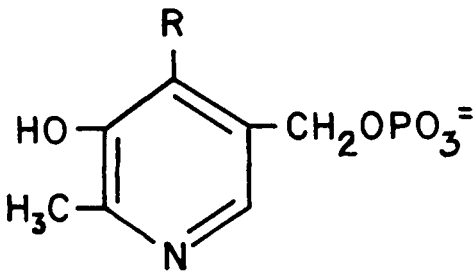
Snell and his associates (1942) were the first to discover that other biologically active forms of the vitamin, pyridoxal and pyridoxamine, exist besides pyridoxine. The structures of pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM) and their respective phosphorylated forms (PNP, PLP and PMP) are diagrammed in figure 1. The International Union of Pure and Applied Chemists - International Union of Biologists Commission on Biological Nomenclature recommends that the term vitamin B_6 includes all 3-hydroxy-2-methylpyridine derivatives that exhibit the biological activity of PN (IUPAC-IUB, 1970).

Function

Pyridoxamine phosphate and pyridoxal phosphate are the two coenzyme forms of the vitamin. The former functions as a coenzyme only in transamination reactions (Snell, 1964). However, the latter, pyridoxal phosphate, "holds an exceptional place among the coenzymes with regard to both the unparalleled diversity of its catabolic functions and to their paramount significance in biochemical transformations of amino acids and in the integral pattern of nitrogen



5-Hydroxymethyl, 3-hydroxy, 2-methylpyridyl



5-Methyl, 3-hydroxy, 2-methylpyridyl, 5'-phosphate

COMPOUND	R
Pyridoxine (PN) Pyridoxine Phosphate (PNP)	CH_2OH
Pyridoxal (PL) Pyridoxal Phosphate (PLP)	CHO
Pyridoxamine (PM) Pyridoxamine Phosphate (PMP)	CH_2NH_2
4-Pyridoxic Acid (4-PA) 4-Pyridoxic Acid (4-PAP)	$COOH$

Figure 1. The free and phosphorylated forms of vitamin B_6 and its metabolites.

metabolism" (Braunstein, 1960). It is involved in transamination, racemization, decarboxylation, cleavage dehydration, desulfhydration and anabolic reactions (Sauberlich and Canham, 1973). The reversible formation of alanine and aspartic and glutamic acids from their respective keto acids, by transamination reactions, is an important link between carbohydrate and protein metabolism. Pyridoxal phosphate transaminases are also indirectly involved in the synthesis of purine and pyrimidine bases, porphyrins, flavins, pteridines and cobalamines. Decarboxylation reactions utilizing PLP as a coenzyme, lead to the synthesis of amines such as taurine, serotonin, histamine, γ-amino butyric acid, γ-amino levulenic acid and dopamine.

In contrast to its coenzyme role in amino acid metabolism, PLP has a structural or conformational role in glycogen phosphorylase (Black, Guirard, and Snell, 1978, 1979). PLP plays a secondary role in lipid metabolism (Sauberlich and Canham, 1973).

Absorption

The absorption of vitamin B_6 occurs primarily in the jejunum. When rats were injected with 0.05 mg of ^{3}H-PN directly into either the upper jejunum, upper ileum, or colon, the percentage of the radioactive dose excreted in the urine was 71, 50, and 18% respectively (Booth and Brain, 1962). The jejunum also appears to be the site of absorption in humans (Brain and Booth, 1964). Patients who had undergone resection of all but 4 to 6 feet of jejunum were given an oral dose of 1.0 mg ^{3}H-PN with a simultaneous intravenous dose of 100 mg non-radioactive PN. Absorption, as indicated by the percentage of radioactivity excreted in the urine, was normal or near normal in three out of the four patients. Many studies have been conducted to determine the mechanism of vitamin B_6 absorption. Passive diffusion has been suggested. Even large doses did not saturate the absorption process, as would be expected if a carrier-mediated process was involved, in the two studies by Booth and Brain. In both rats and humans there was a linear relationship between an oral ^{3}H-PN dose

and urinary excretion of radioactivity. However, the doses administered greatly exceeded daily requirements and normal intakes.

Tsuji, Yamada, and Nose (1973) studied the problem using lower concentrations of ^{3}H-PN with ring segments of everted rat intestine. The rate of uptake of radioactive compounds was linear during the first 10 minutes, and then declined. When the initial concentration in the incubation medium ranged from 0 to 4μM ^{3}H-PN, the ratio of the concentration of vitamin B_6 within the ring segments to that in the medium was always greater than one after 1 hour of incubation. From this evidence one might conclude that vitamin B_6 absorption occurs by facilitated or active transport, since it appears to occur against a concentration gradient. However, when Tsuji and his coworkers identified the specific forms of the vitamin within the rings, they found primarily the phosporylated forms. The small intestine contains the enzyme pyridoxal kinase, which can phosphorylate various forms of the vitamin (McCormick, Gregory, and Snell, 1961). A favorable concentration gradient is maintained for PN absorption because it is phosphorylated soon after it is absorbed.

When Tsuji et al. added 4-deoxypyridoxine, an inhibitor of PL kinase, to the incubation medium, the intra-ring PN concentration increased markedly. During the first 5 minutes PN uptake was linear and significantly depressed. Four-deoxypridoxine inhibited PN uptake primarily in the later phase of absorption. However, even with maximum inhibition, 43% of the PN was absorbed. This evidence suggests that 4-deoxypridoxine inhibits the metabolism of PN to PNP, making the PN concentration gradient less favorable for passive diffusion, and thus limiting absorption.

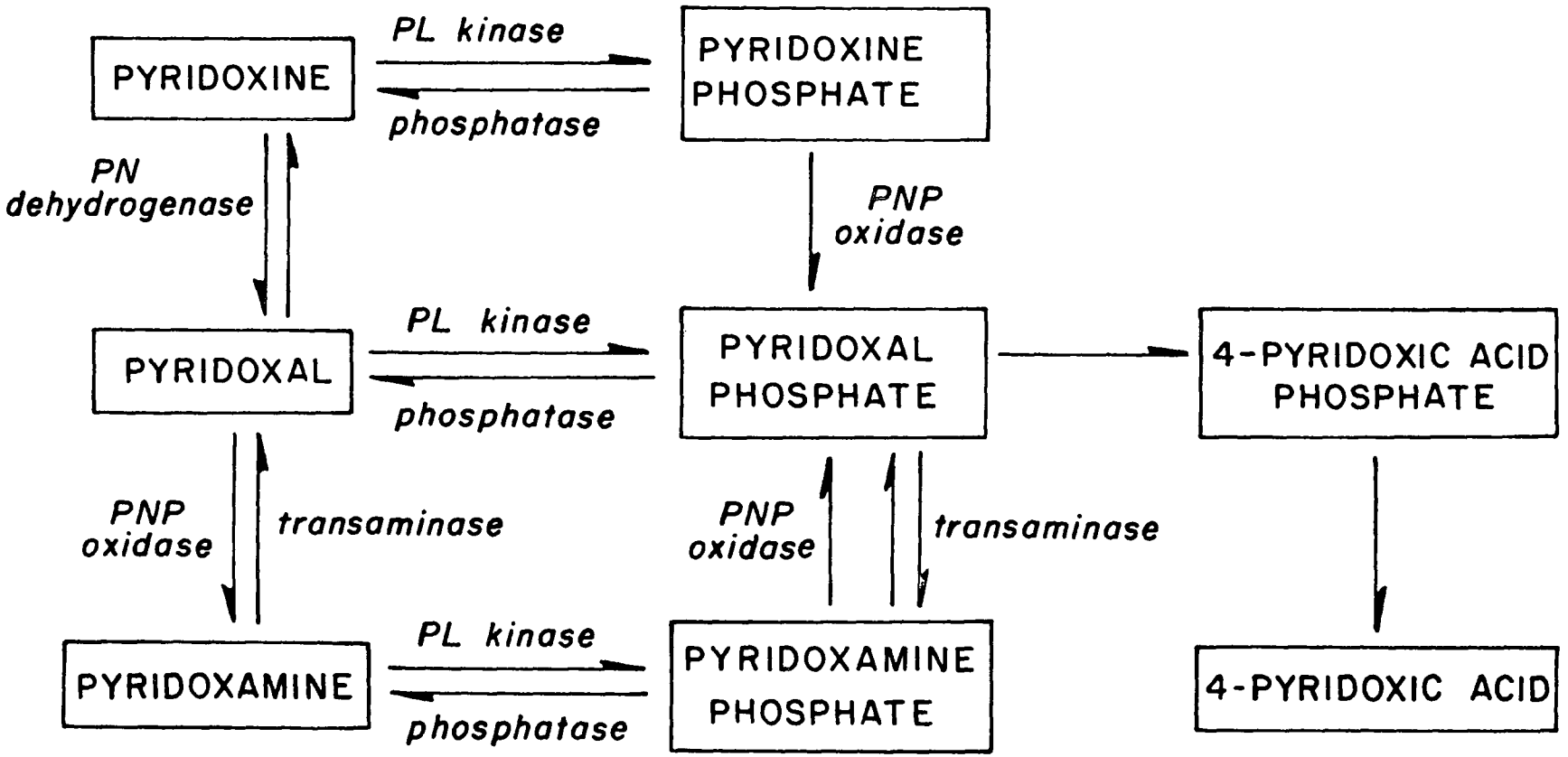
Passive transport of vitamin B_6 has also been suggested by Spencer and Bow (1964), Hajjar and Nassar (1975), Serebro et al. (1966), and Middleton (1977). Middleton (1977) incubated everted jejunal sacs from rats for 4 minutes with solutions of PN ranging from 1 to 10μM. Uptake was linear over this range of concentrations. Extrapolation by linear regression led essentially to inter-

section at the origin. Therefore, saturation kinetics at concentrations less than 1 μM is unlikely. Competetive and metabolic inhibitors such as 4-deoxy-pyridoxine, anoxia, iodoacetamide, and ouabain, when introduced into an incubation medium with 2 μM PN, did not produce a significant decrease in PN uptake. Although an experimental model such as this has been used to show saturation kinetics in the absorption of thiamin (Hoyumpa, 1975), this study provides no evidence for a carrier-mediated absorption process with vitamin B_6.

The bioavailability of vitamin B_6 from a natural and synthetic source was compared in a study by Nelson and co-workers (1976). They performed triple lumen perfusion in the human proximal jejunum. The perfusion substances included orange juice; synthetic orange juice; a synthetic vitamin B_6, glucose, and saline solution; and a synthetic vitamin B_6 and saline solution. Each solution contained 15 μg vitamin B_6/100 ml. The total amount of vitamin B_6 absorbed from each solution was 12 $\pm$ 2, 24 $\pm$ 2, and 40 $\pm$ 7 μg/hr, respectively. The authors suggest that vitamin B_6 absorption is lowest in the orange juice due to an association of the vitamin with a molecule which has a molecular weight greater than 3,000. They cautioned that conclusions about absolute nutrient bioavailability cannot be drawn from this type of experiment because the vitamin in orange juice may be completely absorbed as it travels the entire length of the intestine. But if the rate of absorption is slow and the transit time through the intestine is decreased, total absorption may be affected.

Metabolism

The three free forms of vitamin B_6 are widespread in nature. PL and PM are prevalent in animal products while PN is the predominant form in vegetable products. In man and higher animals all three forms can be converted to the coenzyme forms through enzymatic pathways (Sauberlich and Canham, 1973). (Refer to figure 2). Pyridoxal kinase and pyridoxine phosphate oxidase are two PLP-synthesizing enzymes. The oxidase catalyzes the oxidation of either PMP or PNP to PLP Wada and Snell(1961). PL kinase phosphorylates each of the



Figures 2. The metabolic pathway of vitamin B_6 and its metabolites. Adapted from Sauberlich and Canham (1973) and Contractor and Shane (1970b).

three forms of vitamin B_6. Adenosine triphosphate is the preferred phosphorylating agent. Kinase activity is highest in the brain, kidney, spleen and liver of rats, and lowest in muscle (McCormick, et al., 1961).

Although most tissues have some kinase activity, the liver appears to be the primary source of plasma PLP. Both kinase (McCormick et al., 1961) and oxidase (Wada and Snell, 1961) activity is high in the liver. Lumeng, Brashear, and Li (1974) injected dogs with 1 mg PN/kg body weight. The mean rate of plasma PLP increase was 23 ± 2.8 ng/ml/hour in controls versus only 0.8 ± 2.2 ng/ml/hour in hepatectomized animals. Neither bilateral nephrectomy nor resection of stomach, spleen and intestines significantly altered the control response to PN loading. Similar results were obtained when PL was injected.

Johansson, Lindstedt and Tiselius (1968) intravenously injected mice with 20 μg $^{3}H_8$-PN. Labeled PN disappeared rapidly from the liver. PNP was at a maximum 10 minutes after injection, and then declined rapidly. PLP increased continuously, comprising 70% of the recovered isotope after 60 minutes. The phosphorylated forms of the vitamin appeared much more slowly in the carcass. Similar results were reported by Colombini and McCoy (1970). These authors also noted that PMP synthesis was slow yet continuous in all tissues of mice intravenously injected with 4,5-^{14}C-PN. After 1 hour PMP and PLP were the major vitamin forms present. Eventually PMP levels equalled PLP levels. Since PM synthesis was minimal, the authors suggest that PMP is formed from PLP via transamination, rather than through the phosphorylation of PM.

A more recent study by Johansson, Lindstedt and Tiselius (1974) further elucidated the metabolism of vitamin B_6. Tritium labeled PM, PNP, or PLP was intravenously injected into mice. In the liver, equilibrium was reached within 1 to 2 hours. Regardless of the form originally injected, 60 to 70% of the recovered isotope appeared as PLP, 20 to 30% as PMP, and less than 10% as PM, PNP and PL. The authors propose that PLP is synthesized directly from PMP or PNP since there was a rapid increase in PMP and PLP in both liver and carcass after the injection of PM. In addition, the disappearance of PNP in both liver

and carcass was immediately followed by the appearance of PLP. The pathway for the interconversion of the different forms of vitamin B_6, as shown in figure 2, has been suggested by the work of these and various other researchers.

Vitamin B_6 Metabolism in the Red Blood Cell

In vivo and in vitro experiments on human blood samples were conducted by Anderson and co-workers (1971) to study the metabolism of PN within the red blood cell. In an in vivo experiment with one subject, blood samples were drawn at intervals after a 50 mg oral dose of PN- HCl. At 10 minutes there was an increase in Lactobacillus casei-active vitamin B_6 in red blood cells, indicating that PN had been absorbed by the cells. The activity increased rapidly to a peak at 1 hour, to a second unexplained peak at 2 hours, and decreased. L. casei responds to PL and PLP.

In in vitro studies by this group, ^{3}H-PN was incubated with whole blood. After 1 minute, 7% of the PN had been converted to L. casei-active vitamin B_6. All of the activity was found in the erythrocytes. By 40 minutes all of the PN taken up had been converted to the L. casei-active forms. After 90 minutes, 100% of the ^{3}H-PN had been taken up by the cells. In both the in vitro and in vivo experiments there was an increase in L. casei activity in plasma after the appearance of activity in erythrocytes. The authors suggest that within the erythrocytes, PN is converted to PNP by the enzyme PL kinase. PNP oxidase then catalyzes the synthesis of PLP from PNP. Gradual cleavage of PLP by a phosphatase then allows PL to leave the red blood cell and enter the plasma.

The unphosphorylated forms of the vitamin appear to be more readily transported across cell membranes than the phosphorylated forms (Tiselius, H. G., 1973; Anderson, et al., 1971). PLP in plasma is strongly bound to albumin, while PL is only loosely associated with protein (Anderson, Newmark and Rawlins, 1974). This evidence, as well as the work by Lumeng and associates (1974), suggests that the binding of PLP by albumin prevents entry of the vitamin into the

red blood cell. This may serve a protective or regulatory function since 0.4 to 8.0 nM PLP inhibits the activity of some red cell glycolytic and pentose phosphate shunt enzymes (Srivastava and Beutler, 1972).

Blood Vitamin B_6 Levels

In a study of seven healthy male subjects fed an ad libitum diet the plasma vitamin B_6 levels were three times greater than the levels in red blood cells, on a volume basis (Baker et al., 1969). Three minutes after intravenous injection of 35 mg PN-HCl as part of a multivitamin mixture, the concentration of vitamin B_6 in the red blood cells had increased 24 times and was 3 times greater than the concentration in the plasma. Both levels gradually declined. At 60 minutes the two levels were approximately equal. After 24 hours the vitamin B_6 concentration in both plasma and erythrocytes was still elevated, but the original three to one ratio was virtually restored.

Except for PN and PNP, Contractor and Shane (1968 and 1970) found all forms of the vitamin present in blood. PM was the form in highest concentration in both male and female subjects in the above two studies. However, Kelsay, Baysal, and Linkswiler, (1968) found PL but no PM or PN in the blood of 11 male subjects given 150 or 54 g protein/day in diets containing 0.16, 0.76, or 1.66 mg vitamin B_6/day. At these intake levels the blood vitamin B_6 ranged from 0.14 $\pm$ 0.2 to 0.72 $\pm$ 0.28 μg/100 ml. When a 50-mg oral dose of PN was given an each of two consecutive days, blood samples contained 0.5 to 0.8 μg PN/100 ml, 4 to 5 μg PL/100 ml and no PM. Before microbiological assay with Saccharomyces uvarum the blood samples were autoclaved in 0.1 N HCl for 5 hours. Since this treatment cleaves the phosphate bond, the above figures reflect both the unphosphorylated and phosphorylated forms of the vitamin.

The effect of vitamin B_6 depletion on blood vitamin levels was also examined in a similar study by Baysal, Johnson, and Linkswiler (1966). Six male subjects consumed a diet containing 100 g protein and 0.16 mg vitamin B_6. They were given a supplement of either 0.6 or 0.9 mg PN when vitamin B_6 deficiency developed as indicated by a disturbance in tryptophan metabolism. After 25 days

of depletion no vitamin B_6 was found in the blood. Marginal supplementation resulted in slight but detectable increases in blood vitamin B_6 levels in four of the subjects. The PLP levels also decreased during depletion, but did not increase with supplementation.

In 15 control and hyperactive children, aged 2 through 19 years, receiving a normal diet, blood PLP was equally distributed between erythocytes and plasma (Bhagavan, Coleman and Coursin, 1975). While other forms of the vitamin fluctuated considerably, blood PLP levels remained relatively constant in four female subjects during the course of one menstrual cycle (Contractor and Shane, 1968). Plasma PLP levels in 94 control subjects also remained remarkably constant over a period of time up to 6 months long. However, the variation among individuals ranged from 5.0 to 26.3 ng PLP/ml plasma. Plasma PLP concentration decreased as a function of age in both control and alcoholic subjects (Lumeng and Li, 1974).

The relative stability of blood PLP values, compared to other forms, suggests that PLP is a good indicator of vitamin B_6 status. A study by Lumeng and Li (1978) has further substantiated this role. For 9 weeks weanling rats were fed ad libitum a liquid, purified diet which supplied 0, 4, 12, 24, or 100 μg PN/day. The rate of growth increased in PN intake, reaching a maximum with 24 μg PN/day. Liver and brain PLP concentration increased with PN intake and reached a maximum with 12 μg PN/day. In contrast, plasma and skeletal muscle PLP levels increased with intake but never reached a maximum. Plasma and skeletal muscle PLP was more easily depleted when intake decreased than was brain or liver PLP. It has been suggested that glycogen phosphorylase in skeletal muscle may serve as a reservoir for vitamin B_6 (Krebs and Fischer, 1964; Black, Guirard and Snell, 1978, 1979). Since Lumeng and Li (1978) found a strong, linear, positive correlation between PLP in plasma and skeletal muscle, they suggest that plasma PLP reflects both vitamin B_6 nutriture and body stores.

Urinary Excretion of Vitamin B_6

In urine, vitamin B_6 is excreted in several forms. According to Sauberlich et al. (1972) urinary vitamin B_6 is primarily PL followed by PM. Pyridoxine and the phosphorylated forms are found in small amounts. Contractor and Shane (1968 and 1970a) found primarily PM, with lesser amounts of PN and PL. The phosphorylated forms were detected either in small amounts (Contractor and Shane, 1968) or not at all (Contractor and Shane, 1970a).

The two major metabolites of vitamine B_6, 4-pyridoxic acid (4-PA) and 4-pyridoxic acid phosphate (4-PAP), are also excreted in the urine. The structures of these compounds are illustrated in figure 1. Johannson et al. (1966) administered 400 μg ^{3}H-PN intravenously to two subjects and 250 μg ^{3}H-PN orally to one subject. Isotopic studies of the urine revealed that 4-PA constitutes 20 to 40 % of the excreted isotope. This experiment, however, was conducted before 4-PAP was isolated and characterized from the urine of male Wistar rats (Contractor and Shane, 1970b). It was known to be a chemical oxidation product of PLP, but until this time had not been found in animal tissues or urine. Results of this study suggest that PLP is first converted to 4-PAP and then to 4-PA before removal from tissues.

Many authors have shown that urinary excretion of vitamin B_6 and 4-PA decreases rapidly during vitamin B_6 depletion. (Baysal et al., 1966; Kelsay et al., 1968; and Linkswiler, 1967). During the depletion period in all of these studies the vitamin B_6 intake was 0.16 mg/day. Excretion of vitamin B_6 in the urine of male subjects receiving 55 or 100 g protein/day decreased more slowly than excretion of 4-PA (Linkswiler, 1967). No 4-PA appeared in the urine after 24 days of the vitamin B_6-deficient high-protein diet. In a similar study by Baysal et al. (1966) there were significant increases in both urinary vitamin B_6 and 4-PA when supplements of 0.6 or 0.9 mg vitamin B_6 were given after 27, 36, or 40 days of depletion. However, the values remained far below pre-depletion levels.

Another study which determined urinary levels of vitamin B_6 and 4-PA during depletion and repletion was conducted by Baker et al. (1964). A liquid formula diet containing 0.06 mg vitamin B_6 and either 30 or 100 g protein/day was fed to eight male subjects. After a deficiency state developed, as determined by tryptophan load tests, 1.0 mg vitamin B_6/day for two weeks and then 1.5 mg vitamin B_6/day were given as a supplement. In both the high and low protein groups, urinary vitamin B_6 excretion correlated well with levels of vitamin intake. Both urinary vitamin B_6 and 4-PA are often used as an indicator of recent vitamin B_6 intake, but are not reliable measures of vitamin B_6 status.

Fecal Excretion of Vitamin B_6

Research on the fecal excretion of vitamin B_6 has been limited. In infants it ranges from 0.15 to 0.31 mg/day (Møller, 1951). Adult fecal excretion of the vitamin ranged from 0.7 to 0.9 mg/day in a study by Linkswiler and Reynolds (1950). Excretion did not significantly increase when the basal intake of 0.76 mg PN/day was increased by 15 mg. A later study by Levy (1969) showed that the combined excretion of PN, PM and PL was 0.33 to 0.81 mg/day when the vitamin B_6 intake ranged from 2.12 to 3.06 mg/day. Fecal excretion was also in this range in a recent study by Perera (1977).

Fiber

In recent years fiber has received much attention in both the popular and scientific press regarding its role in maintaining health and preventing disease. A low fiber diet is purported to be associated with a wide variety of disorders including ischemic heart disease (Trowell, 1972), appendicitis, diverticular disease of the colon, gallbladder disease, colon cancer, varicose veins, diabetes mellitus and obesity (Kelsay, 1978). Such claims, often based on epidemiologic evidence, have prompted detailed scientific investigations into the composition, properties, and mode of action of fiber, and the effects of fiber on human

health, intestinal absorption, and bowel function.

A variety of terms are used in reference to fiber, including bulk, roughage, plant fiber, crude fiber, dietary fiber and unavailable carbohydrate. All of these terms have slightly different meanings, making the nomenclature in this field ambiguous and complex. Most tables of food composition only give values for "crude fiber", a term which has been officially in use since 1887 (Mendeloff 1977). This term has its limitations since it does not accurately reflect all of the plant components that are indigestible by human digestive enzymes. These components include the structural polysaccharides of the plant cell wall such as hemicelluloses, pectic substances and cellulose, and the non-carbohydrate, lignin (Soutgate et al. 1976a). The method for the determination of crude fiber hydrolyzes 80% of the hemicellulose, 50 to 90% of the lignin, and 20 to 50% of the cellulose (Kelsay, 1978).

Dietary fiber, a more meaningful term than crude fiber, has recently been introduced (Trowell, 1977). It is defined as the sum of the storage and structural plant polysaccharides and lignins that are not digested by the human gastrointestinal tract. The measurement of dietary fiber in foods involves several analytic procedures (Southgate, 1976). Values for dietary fiber are two to five times greater than those obtained for crude fiber.

The composition and physical properties of dietary fiber varies with the plant depending upon the maturity of the plant and the conditions under which it was grown (Southgate, 1976). Some fibers are completely insoluble while others are totally soluble in the human intestine. Some undigested fibers are metabolized by intestinal bacteria while others resist this fermentation (Mendeloff, 1977). This variation makes it impossible to generalize about the physiologic role of all fiber. Even though many foods, such as cereals, fruits, nuts, legumes, vegetables, and some processed foods contribute to the quantity and quality of fiber in the diet, the present discussion is restricted to the effects of wheat bran on intestinal absorption and function.

Wheat Bran

Bran is the outside coat of the wheat kernel which is removed during the milling process. This coat consists of various layers, including the epidermis, hypodermis, seed coat, and aleurone layer (Saunders, 1978). The American Association of Cereal Chemists Food Fiber Committee developed a certified food grade wheat bran in response to a request by the National Academy of Sciences for a standardized bran source (AACC Food Fiber Committee, 1977). The composition of this bran is given in Appendix table 1.

The laxative effect of bran on bowel function is well documented. In an early study, 11 healthy men were fed a carefully controlled diet to which was added wheat bran or wheat bran treated with weak acid to remove the phytin (Cowgill and Anderson, 1932). There was no difference in the laxative effects of these two treatments. The rate of laxation was inversely related to the amount of fiber ingested per unit body weight. The authors estimated the physiologic roughage minimum to be 90 to 100 mg fiber/kg body weight.

A subsequent study by Cowgill and Sullivan (1932) found wheat bran to be more effective than fruits and vegetables in relieving constipation. Improvement was reported in 107 out of 135 patients suffering from constipation who had added bran to their diets. In 42 of these patients this improvement was corroborated roentgenologically (Streicher and Quirk, 1943).

The Effect of Wheat Bran on Intestinal Transit Time and Stool Weight

More recent quantitative studies on the effect of bran on intestinal transit time in normal subjects have yielded a variety of results. A 29% fall in transit time was observed in 19 subjects who for 22 days replaced wholemeal bread for white bread and added approximately 14 g of wheat bran to their normal low-fiber diets (Payler, 1973). The mean transit time fell from 64.5 to 45.9 hours. In another study six subjects who added 10 g of bran to an unrestricted diet

experienced a 23% decrease in transit time (Findlay, et al. 1974).

These two studies did not involve any type of dietary control besides the addition of fiber during the experimental period. In a controlled study, total dietary fiber was increased from 17 g in the control period to 45 g in the experimental period, by the addition of various amounts of wholemeal bread, bran biscuits, and wheat bran cereal. For all six subjects the mean transit time decreased significantly during the last week of the two 3-week periods from 57.8 to 40.3 hours (Cummings, et al. 1976).

Less consistent results were obtained in a study by Harvey, Pomare, and Heaton (1973). These researchers studied 4 healthy subjects and 16 subjects who had had a cholecystectomy, or suffered from constipation or irritable bowel syndrome. Before and after at least 4 weeks on a high-fiber diet containing wholemeal bread or their usual low-fiber diet supplemented with 30 g bran/day, transit time was measured. Seven subjects experienced no change with increased fiber. Mean transit time increased to 1.72 days in the five subjects whose initial transit time was 1 day. For eight subjects the mean transit time decreased from 3.8 to 2.4 days. Initially only 3 out of 20 subjects had a 2-day transit time. After bran supplementation, 13 subjects had a 2-day transit time. Two other groups of researchers have also observed this variable effect of bran on transit time (Payler et al. 1975; Eastwood et al. 1973). It has been suggested that bran normalizes intestinal transit by lengthening short and shortening long transit times.

Fine and Coarse Wheat Bran

Payler et al. (1975) conducted a double blind trial with first 20 and then 40 g of either fine wheat bran or oatmeal added to a normal diet. Transit time was accelerated in all ten subjects from a mean of 3.3 to 2.5 days with bran but was slowed to 3.6 days with the oatmeal treatment.

Although Payler and his group demonstrated that fine bran influences transit time, Kirwan et al. (1974) found fine bran to be less effective than coarse bran. Twice each day 10 g of fine or coarse bran was given to 13 patients with diver-

ticular disease or constipation. Mean transit time was significantly accelerated in the coarse but not in the fine bran group. When the subjects in the fine bran group were subsequently given a coarse bran supplement, their mean transit time also declined. The authors suggest that this difference in action is due to differences in the water-holding capacity of the two types of wheat bran. The water-holding capacity of the coarse bran, as measured by these authors, was 6.15 g water/g bran while that of the fine bran was only 2.36 g water/g bran. The coarse bran also had a greater percentage of acid detergent fiber and lignin, and a greater cation exchange capacity than the fine bran.

Raw or Cooked Bran

The tremendous water holding capacity of wheat bran appears to influence stool weight as well as transit time. Both of these parameters, as affected by raw or cooked bran, were investigated by Wyman et al.(1976). Each subject was randomly assigned to two control periods and four experimental periods with supplements of either 12 or 20 g raw bran/day or 13.2 or 22 g cooked bran/day. Although there was much individual variation in response, the dose of 20g raw bran/day was the only treatment to produce a significant decrease in transit time or increase in stool volume, as compared with the control. Fecal wet weight and the percentage of water in the stools was not significantly altered by any of the four treatments. Dry weight was significantly increased from 30 to 42 g with 12 g of raw bran and from 30 to 37.6 with 20 g of raw bran. Cooked bran did not effect a significant change. Even though raw bran was consistently more effective than cooked bran in altering stool characteristics and bowel function, the mechanism for this is yet unclear. However, cooking does not appear to affect the water holding capacity of bran (McConnel, Eastwood and Mitchell, (1974).

Numerous other studies have shown that the addition of wheat bran to unrestricted or controlled diets increases stool wet and dry weights (Fantus and Frankl, 1941; Payler, 1973; Fuchs, Dorfman, and Floch, 1976; Eastwood et al. 1973).

Early work by Fantus, Hirschberg, and Frankl (1941) investigated the effects of 1 oz of raw or cooked bran of varying particle size on stool weight. The 200 subjects added the bran to unrestricted diets. In the various treatments 55 to 75% of the subjects experienced an average increase in stool weight from 56 to 98%. The change in stool weight was not dependent upon bran particle size.

A more recent study (Fuchs et al. 1976) with four subjects consisted of a 3-week control period followed first by 3 weeks with a daily 3-oz supplement of processed wheat bran and then another 2 weeks of control. Stool weight was 103 $\pm$ 40 g/day during the first control, 226 $\pm$ 90 g/day during the bran period and 58 $\pm$ g/day during the second control. The percentage of water in stools was constant throughout the experiment. Similar results were obtained when the bran was administered in biscuit form (Baird et al. 1977).

Fecal wet and dry weight/day also increased with the addition of only 10 g of bran to the unrestricted diets of normal subjects (Findlay et al. 1974). In this experiment two stool markers were used to monitor changes in stool water content. Polyethylene glycol (PEG) marks the liquid entrapped in the interstices of dietary residue. Chromium sesquioxide (Cr_2O_3) marks the solid dietary residue and the gel or non-solvent water intimately associated with it. Bran supplementation did not change the concentration of Cr_2O_3 but signigicantly reduced PEG marker by almost 50%. The authors conclude that the increase in stool weight during bran supplementation was related to an increase of solid material and associated non-solvent (gel) water rather than an increase in interstitial water.

The study by Cummings et al.(1976) described earlier in this review, examined the effect of increased fiber on stool weight, water content and ion excretion as well as on intestinal transit time. Fecal weight increased from 79.3 $\pm$ 6.6 to 228.0 $\pm$ 29.9g with the additional fiber. The average increase was 5.3 g of fecal material for each gram of fiber added to the diet. In contrast to the results of the work by Fuchs et al. (1976) and Baird et al. (1977), the percentage of stool water increased significantly with supplemental bran.

The Effect of Wheat Bran on Nutrient Excretion

Cummings and his group are one of a number of researchers that have noted a change in the excretion or absorption of ions or minerals with increased bran intake. The high-fiber diet resulted in a statistically significant increase in the fecal excretion of Ca, Mg, P, Na, K, Cl, fatty acids, and other organic anions. Although the total anion excretion increased, the fecal anion concentration decreased on the experimental diet.

In a study by Eastwood and Mitchell (1976) 16 g bran/day for 3 weeks was added to the normal diets of subjects. Fecal excretion of cations in mmoles/day increased from 2.02 $\pm$ 2.57 to 3.62 $\pm$ 3.70 (Na), from 10.7 $\pm$ 4.43 to 18.66 $\pm$ 5.79 (K), from 76.11 $\pm$ 18.15 to 90.53 $\pm$ 35.90 (Ca), and from 35.75 $\pm$ 8.65 to 49.84 $\pm$ 20.48 (Mg). The increase in Ca excretion was not significant. The authors suggest that bran acts as a polyfunctional cation exchanger within the intestinal tract to increase excretion and decrease absorption of selected nutrients.

In vitro experiments have been performed to examine more closely the absorption of minerals to wheat bran. Untreated bran or bran treated to remove phytic acid, which is also able to bind minerals, was incubated with a 10 mg Ca/100 ml solution (Reinhold, Ismail-Beiji, and Faradj, 1975). Dephytinization lead to an increase in the % of Ca bound to the bran. The increase was proportional to the increase in fiber concentration that accompanied the removal of phytate. When fiber was destroyed by an HCl treatment, Ca binding decreased. Cellulose also binds Ca but with less tenacity than bran. The adsorption of Fe^{+3} and Zn also increased with the dephytinization of bran. When phytic acid was returned to the dephytinized mixtures, the % of bound Zn declined so that it approximated the percentage of bound Zn in untreated bran. Zinc adsorption was greatest at pH 6.8 to 7.0, and least at the lowest pH evaluated, 5.0. Because of these findings the authors suggest that Zn adsorption involves an ion exchange process.

Another in vitro study was conducted by Ismail-Beiji et al. (1977). Wheat bran was fractionated and the pectin removed. Lignin and three hemicellulose

fractions were separated and treated with alkali. The alkali treatment may somewhat enhance fiber's metal binding capacity by liberating polar groups. The whole bran and the various fractions were tested for their capacity to bind Zn from a 1.43 g Zn/ml solution at pH 6.8. Whole bran binds 82.5 ± 3.0% lignin, 85.6 ± 1.3%; hemicellulose fraction A, 87.1 ±3.9%; fraction B, 35.7 ± 2.4%; and fraction C, 82.1 ± 9.1% of the Zn. Bran and hemicellulose fraction A were tested for their capacity to bind Fe^{+2} at various concentrations. Fraction A was more effective than whole bran at all concentrations tested. The % of Fe^{+2} bound decreased as the Fe concentration increased.

The effect of increased levels of fiber in the diet on the excretion of vitamin B_6 and 4-PA was examined by Perera (1977). In this study nine healthy male subjects consumed 570 to 600 g whole wheat bread, white bread to which vitamin B_6 was added (vitamin B_6 white bread), or white bread plus a daily oral supplement of 0.81 mg PN (plain white bread). Each type of bread, in addition to a constant diet, was consumed for 7 days. The breads contained 0.21, 0.20, and 0.06 mg vitamin B_6/100 g bread, respectively. The vitamin B_6 content of the three diets was 1.58, 1.56, and 1.54 mg vitamin B_6, respectively. Fecal vitamin B_6 was significantly elevated in subjects receiving the whole wheat bread diet. Expressed as % of intake, fecal vitamin B_6 was 45.2 ± 6% with whole wheat bread, 29.4 ± 0% with vitamin B_6 white bread, and 28.6 ± 6% with plain white bread. Urinary levels of the vitamin were not significantly different with any of the three breads. Urinary 4-pyridoxic acid was 3.40 ± 1.03 μmoles/24 hours with whole wheat, 3.89 ± 0.79 μmoles/24 hours with vitamin B_6 white bread and 3.91 ± 0.95μmoles/24 hours with plain white bread.

As part of the same experiment Peffers (1977) investigated the plasma PLP and vitamin B_6 levels during consumption of the various types of bread. Plasma PLP concentration was 7.6 ± 2.8 ng/ml with whole wheat bread, 8.3 ± 3.2 ng/ml with vitamin B_6 white bread, and 8.5 ± 2.7 ng/ml with plain white bread. Plasma total vitamin B_6 was only 7.4 ± 2.1 ng/ml with whole wheat bread, while it was 8.0 ± 1.8 ng/ml with both types of white bread.

MATERIALS AND METHODS

Subjects were recruited through advertisements and personal interviews. An initial chemical screen[1] was done on prospective subjects to determine the levels of the following components of serum: triglycerides, lactate dehydrogenase, glutamate oxaloacetate transaminase, glutamate pyruvate transaminase, alkaline phosphatase, bilirubin, albumin, cholesterol, urea nitrogen, glucose, globulins, the albumin to globulin ratio, P, Ca, Cl, Na, and K (see Appendix table 2). A xylose absorption test was administered to measure intestinal absorption (Henry, Cannon, and Winkleman, 1974). The ten healthy men 21 to 34 years of age and of normal weight and physical activity were selected to participate in this experiment based on the results of these tests and the initial interview. Their ages, heights, and weights are listed in table 1. The purpose and design of the experiment was fully explained to all subjects. Each one signed an informed consent form before the study began. The experiment was approved by the O.S.U. Human Subjects Committee on December 5, 1975.

Except for meals, the subjects continued their normal daily activities throughout the 59-day study. The subjects consumed no alcoholic beverages, drugs, or vitamin supplements, except the vitamin B_6 solution that was added to the plain muffins and the 2.5 mg PN-HCl loading dose on 11/22/77. All of their meals were prepared, served, and consumed in the metabolic unit of the O.S.U. Department of Foods and Nutrition under the supervision of a nutritionist. The study consisted of a 4-day adjustment period followed by two 18-day and one 19-day experimental period, in that order. All subjects consumed diet A (table 2) during the adjustment period and the experimental diet (table 3), with or without supplemental wheat bran, during the remaining 55 days of the study. Both diets were nutritionally adequate. The composition of the diets is given in table 4.

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1. Performed at Good Samaritan Hospital, 3600 Samaritan Drive, Corvallis, Oregon, using the Hycel Super 17, Hycel Incorporated, Houston, Texas.

Table 1. The vital statistics of the subjects participating in the study.

Subject No.	Nationality	Birthdate	Age[a] (years-months)	Height (cm)	Weight (kg) Begin	Weight (kg) End
1	American	11/29/53	23 - 10	176.5	61.5	64.3
2	Peruvian	1/1/48	29 - 9	160.7	61.5	61.8
3	American	11/19/47	29 - 10	167.6	55.6	58.6
4	Peruvian	7/10/43	34 - 2	169.5	71.0	73.7
5	American	11/13/56	20 - 11	193.7	87.3	86.9
6	American	3/24/51	26 - 6	178.4	95.5	93.2
7	American	3/23/53	24 - 6	181.0	76.9	76.5
8	American	3/21/49	28 - 6	184.2	65.1	67.8
9	American	12/18/53	23 - 9	171.4	48.0	50.9
10	American	5/23/55	22 - 4	179.4	67.9	67.6

a. Their ages at the beginning of the study.

Table 2: DIET A, consumed during the adjustment period.

Breakfast:	Weight (grams)
Orange juice, reconstituted frozen	125
Cornflakes	30
Half and half	125
Hard cooked egg (1)	
Bread, white enriched	25
Banana	30
Margarine[a]	75
Lunch:	
Milk, whole	250
Bread, white enriched	50
Bologna	30
Oatmeal cookie (2)	
Raisins	40
Chocolate bar	34
Carrots	100
Dinner:	
Milk, whole	250
Casserole:	
Ground beef, raw	75
Macaroni, raw	60
Green pepper, fresh	25
Dehydrated onion	10
Tomato paste, canned	40
Bread, white enriched	50
Peaches, canned	90
syrup	30
Vanilla ice cream	75

a. Fleischmann's Corn Oil Margarine. Standard Brands, Inc., New York, 10022

Table 3. The experimental diet, consumed throughout the 55-day experiment.

		Weight (g)
Breakfast:		
	Bread, enriched white	50
	Orange juice, reconstituted frozen	230
	Grapefruit sections, canned	100
	Syrup	20
	Nan-fat dry milk solids	22.5
	Wheat Hearts[a], dry	30
	Tomato juice, canned	30
	Margarine[b]	100
	Cocoa mix[c], dry	30
Lunch:		
	Cheese, cheddar	30
	Carrots, raw	25
	Bread, enriched white	50
	Tomato juice, canned	100
	Raisins	50
	Raspberries, frozen	60
Dinner:		
	Ground beef, raw	120
	Green beans, canned	100
	Juice	10
	Pears, canned	100
	Syrup	20
	Rice, dry	45
	Graham crackers	50

Table 3. (continued)

BRAN DIET

Experimental diet plus:	Weight (g)
Wheat bran[d]	15
Muffin mix, dry	85

NON-BRAN DIET

Experimental diet plus:

Muffin mix[e], dry, with 0.186 mg PN[f]

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a. Wheat Hearts[R], General Mills, Minneapolis, Minnesota
b. Fleishmann's Corn Oil Margarine, Standard Brands, Inc., New York 10022
c. Master mix recipes for cocoa and muffins given in Appendix Table 3.
d. Five grams of wheat bran were incorporated into and baked with the cereal at breakfast, the muffins at lunch, and the meat at dinner. The bran groups ate all three muffins at lunch.
e. The non-bran groups ate one muffin at each meal.
f. Added as 0.226 mg PN-HCl. Lot 501655 Calbiochem, San Diego, California.

Table 4. Nutrient content of adjustment and experimental diets, and of 15 g wheat bran.

Nutrient	ADJUSTMENT Amount[a]	ADJUSTMENT %RDA[b]	EXPERIMENTAL Amount[a]	EXPERIMENTAL %RDA[b]	WHEAT BRAN(15g) Amount[c]	WHEAT BRAN(15g) %RDA[b]
Protein (g)	80.5	144	81.3	145	2.15	3.8
Calories (kcal)	2424	84	2622	97	-	-
Fat (g)	93.2	-	102.3	-	0.78	-
Carbohydrate (g)	323	-	359.8	-	-	-
Calcium (mg)	1163.7	145	1186.3	148	18	2.2
Phosphorus (mg)	1417.7	177	1636	205	156	19.5
Iron (mg)	14.97	150	18.39	184	1.83	18.3
Sodium (mg)	1924	-	3249.5	-	15	-
Potassium (mg)	3443.1	-	3047	-	207	-
Vitamin A (i.u.)	15,668	470	8314	250	-	-
Thiamin (mg)	1.77	126	1.57	112	0.12	8.5
Riboflavin (mg)	2.36	148	1.92	120	0.06	3.8
Niacin (mg)	15.68	87	18.02	100	3.13	17.4
Vitamin C (mg)	132	293	197	438	-	-
Vitamin B_6[d] (mg)	1.59	74	1.62	86	0.186	8.7
Neutral detergent fiber[e] (g)	3.63	-	6.6	-	7.1	-

a. Calculated from Agriculture Handbook No. 8. Composition of Foods. Agriculture Research Service, USDA, Washington D. C. 1976.
b. Percent of the recommended dietary allowance for an adult man (National Academy of Sciences, 1974).
c. Calculated from American Association of Cereal Chemists (1976) see Appendix table 1.
d. Determined by microbiological method (AOAC, 1975) using Saccharomyces uvarum as the assay organism.
e. Determined by method of Van Soest and Wine (1967) by Yu (1979).

The men were randomly assigned to one of two groups. The three periods and the two groups of men were organized in a switch-back design (figure 3). Group 1, subjects 1 through 5, consumed 15 g wheat bran/day in addition to the experimental diet during the first and third periods. Group 2, subjects 6 through 10, ate the additional 15 g wheat bran/day only during the second period. The vitamin B_6 intake was 1.59 mg/day during the adjustment period, 1.69 mg/day during the bran periods, and 1.66 mg/day during the non-bran periods. Dietary neutral detergent fiber was 3.63, 13.7, and 6.6 g/day during the adjustment, bran, and non-bran periods, respectively (Yu, 1979). On 11/22/77, the second to the last day of the third period, all subjects received a 2.5 mg oral loading dose of PN-HCl[2] in 10 ml of 1.0% acetic acid after they had consumed only their cereal at breakfast. They then ate the remainder of their breakfast. The results of the loading dose will be presented elsewhere.

The bran used in this study was the American Association of Cereal Chemists' certified wheat bran.[3] During the bran periods 5 g of wheat bran was consumed by each subject at each of the three meals. The bran was incorporated into and baked with the breakfast cereal, the muffins at lunch, and the meat at supper. The wheat bran group ate three muffins containing a total of 5 g of wheat bran at lunch. The non-bran group ate one plain muffin at each meal. The muffins were mixed in individual portions and baked daily for consumption the following day. Before baking the amount of vitamin B_6 in 15 g of bran, 0.186 mg PN[4], was added as a solution to the three plain muffins. Master mixes for the muffins and the cocoa were prepared before the study began. Recipes for these master mixes are presented in Appendix table 3.

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2. Lot 501655, Calbiochem, San Diego, Califomia.
3. AACC Certified Food Grade Wheat Bran, RO7-3691, St. Paul, Minnesota The composition of this bran is given in Appendix Table 1.
4. Added as 0.226 mg PN-HCl, Lot 501655, Calbiochem, San Diego, Calif.

PERIOD	A	1	2	3
DURATION days	4	18	18	19
dates	9/26 - 9/29	9/30 - 10/17	10/18 - 11/4	11/5 - 11/23
TREATMENT Group 1 (Subjects 1-5)	DIET A	EXPERIMENTAL DIET BRAN	NON-BRAN	BRAN 11/22-2.5 mg PN-HCl
Group 2 (Subjects 6-10)	DIET A	EXPERIMENTAL DIET NON-BRAN	BRAN	NON-BRAN 11/22-2.5 mg PN-HCl
URINE COLLECTION	DAILY			
FECAL MARKER	9/26	EVERY 3 DAYS BEGINNING ON 9/30		
FECAL COMPOSITES		3 -DAY COMPOSITES		
BLOOD DRAWN	9/26	EVERY 3 DAYS BEGINNING ON 9/30 11/22 - BLOOD DRAWN 0, $\frac{1}{4}$, 1, 3, 5 HOURS AFTER LOADING DOSE		
FOOD COMPOSITES		WEEKLY		

Figure 3. Experimental design and schedule of sample collection. Diet A is presented in table 2. The experimental diet is presented in table 3.

Toasted bread was allowed only at breakfast. The alloted portion of margarine was consumed during the three meals. In order to help maintain body weight subject 5 was given 100 g margarine/day as of 9/30/77 and subject 10 was given 95 g margarine/day as of 10/6/77. Vitamin B_6-free foods such as 7-UP[5], hard candy, sugar, honey, mustard, coffee, and tea were eaten ad libitum. Each subject recorded the amounts of these foods consumed and weighed himself each day before breakfast. The subjects were encouraged to maintain constant body weights by regulating their intake of the calorie-rich "free" foods (7-UP, hard candy, sugar, and honey) in accordance with body size, activity, and metabolic rate.

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5. Seven-Up Enterprises, St. Louis, Mo. 63105

Sample Collection and Preparation

Daily 24-hour urine specimens were collected under toluene and refrigerated. Urine was analyzed immediately for total vitamin B_6 and creatinine, or stored frozen for later determination of 4-pyridoxic acid. Completeness of urine collection was monitored by the determination of creatinine. Refer to figure 3 for a schedule of sample collections.

Feces were collected in air-tight plastic bags. A fecal marker, 50 mg F.D. & C Brilliant Blue Dye No. I mixed with 200 mg methylcellulose, was given to the subjects in a gelatin capsule on the first day of the adjustment period, on the first day of the first experimental period, and every 3 days after that. Three-day fecal composites were mixed and frozen until analyzed for total vitamin B_6 content.

A licensed medical technologist collected blood samples from the antecubital vein into heparinized vacutainer tubes. Blood was drawn from fasting subjects before breakfast on the first day of the adjustment period, and every third day after that. On 11/22/77, the next to the last day of period 3, blood was drawn before 2.5 mg PN-HCl was administered at breakfast, and then $\frac{1}{2}$, 1, 3, and 5 hours later. After the hemoglobin content and the hematocrit were determined, the blood was centrifuged at 3200 rpm and at 0° for 30 minutes. Plasma was analyzed immediately for vitamin B_6 or stored at -50° for subsequent determination of PLP. A second chemical screen[1] was performed on the day of the vitamin B_6 loading dose (see Appendix table 2).

Each week a composite of 1 day's diet was made, alternating between the bran and non-bran diets. Samples of the composite were frozen until analyzed for vitamin B_6 content.

Analytical Methods

The vitamin B_6 and PLP determinations were conducted in subdued light to prevent destruction of the vitamin by ultraviolet light. Vitamin B_6 in the diet, plasma, urine and feces were analyzed using the Association of Organic and Analytical Chemists microbiological method (A.O.A.C., 1975) with Saccharomyces uvarum (ATCC No. 9080) as the assay organism. The initial hydrolysis step in this procedure varies with the material to be analyzed. Portions of the food composite or the fecal samples[6] were added to 200 ml 0.44 N HCl, and autoclaved at 15 lbs pressure for 2 hours. After cooling, the pH of the mixture was adjusted to 4.5. The volume was then adjusted to 250 ml with redistilled water and filtered through Whatman No. 1 filter paper.

Before microbiological analysis for total vitamin B_6[7], plasma was added to 10% freshly prepared trichloroacetic acid (TCA) so that the ratio of plasma to TCA was 1 to 5. This mixture was allowed to stand refrigerated for 30 minutes, and then centrifuged at room temperature for 30 minutes at full speed in an International Centrifuge[8]. The supernatant was decanted and autoclaved for 30 minutes at 15 lbs pressure. After cooling, the pH was adjusted to 4.5 with 6 N KOH. The volume was then adjusted to 40 ml with redistilled water. Since treatment with TCA and subsequent autoclaving hydrolyzes phosphorylated forms of the vitamin, microbiological analysis of plasma measures the dephosphorylated as well as the free forms of vitamin B_6. S.uvarum responds only to the free forms of vitamin B_6.

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6. Dietary, urinary, and fecal vitamin B_6 determined by M. Edwards.
7. Plasma vitamin B_6 determined by E. M. Bensen.
8. International Centrifuge, Size 1, Type SB, No. W9601. International Equipment Co., Boston, Mass.

Urine samples were prepared for determination of total vitomin B_6 content by first adding 50 ml of 0.1 N HCl to 10 ml or 1% of the 24-hour urine volume. The mixture was then outocloved for 30 minutes ot 15 lbs pressure. After the pH was adjusted to 4.5 with KOH, the volume was increased to 100 ml with redistilled water and filtered through Whatmon No. 1 filter poper. After hydrolysis of urine, fecal, plosmo, or diet samples, vitamin B_6 determination proceeded using a slight modification of the AOAC method os described by Perera (1977).

Urinary 4-pyridoxic ocid wos determined using a chromatographic and fluorometric procedure (Reddy, Reynolds, and Price, 1958). Creotinine in urine wos determined on o Technicon Autoanalyzer by an automoted modification of the Jaffe reaction (Pino, Benotti, and Gardyna, 1965).[10]

The PLP content of plasma was determined by a radiotrocer, enzyme assay Rose (1974).[11] The procedure was further modified os follows: After deproteinization and centrifugation of the plasmo, 1 ml of the clear supernotant was pipeted into centrifuge tubes containing 0.25 ml of cold 5M potossium ocetate ond 0.05 ml of cold deionized redistilled woter. The final colculations included o dilution foctor which accounted for the decreased omount of somple supernatant. Either Hyamine Hydroxide[12] or NCS[13] was used os the CO_2 tropping ogent.

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9. Technicon Autoonalyzer, Technicon Corp., Torrytown, New York.
10. Urinary creatinine determined by L. Barstow.
11. Some PLP determinations by T. Shultz.
12. Hyamine Hydroxide®, New England Nuclear, Boston, Massachusetts.
13. Nuclear Chicogo Stabilizer®, Nucleor Chicago, Des Plains, Illinois.

Statistics

Statistical analysis of variance (ANOVA) for the switch back design of the experiment was determined for all data.[14] This analysis utilized the system of the Statistical Package for the Social Sciences (SPSS). Students t test for paired data was performed on the urinary and fecal data. Pearson correlation coefficients were also calculated.

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14. Statistical analysis by Jo An Barnes, O.S. U. Statistics Department.

RESULTS

Statistical analysis of the data, based on the switch back design of this experiment, revealed no significant differences between the bran and non-bran treatments for any of the parameters studied. However, these data show consistent trends. And, when the three periods are considered separately, both individual and group data show similar patterns. There were statistically significant differences between the urinary vitamin B_6 ($p<0.05$), plasma vitamin B_6 ($p<0.05$), and plasma PLP ($p<0.01$) values for the two groups. (See figures 5, 8, 9, and table 5). This difference was not related to treatment. All individual and group data are presented in the appendix. For statistical analysis and the following discussion, data from the last half of each period for each parameter were averaged to allow for adaptation of the subjects to the treatment.

Total Vitamin B_6 in Feces

The mean fecal total vitamin B_6, expressed as % of intake, decreased for all subjects from 37.2 $\pm$ 11.0% with bran to 34.4 $\pm$ 8.7% without bran. Individual and group means for each period are presented in figure 4 and table 5. In group 1 the subjects' mean fecal vitamin B_6 was 39.7 $\pm$ 8.5% during the first bran treatment, decreased to 34.9 $\pm$ 8.3% during the non-bran treatment, and then increased to 37.2 $\pm$ 15.0% during the second bran treatment. In group 2 the subjects' mean fecal vitamin B_6, also highest during the bran treatment, was 34.0 $\pm$ 10.8%, 35.8 $\pm$ 6.9%, and 34.2 $\pm$ 6.5% without, with and without supplemental wheat bran, respectively. In both groups there was much individual variation in the response to bran supplementation. Fecal vitamin B_6 was consistently higher with than without bran in subjects 3, 5, 7, 9, and 10. Subject 5 had a marked drop in fecal vitamin B_6 from the bran to the non-bran period, followed by an even greater rise during the second bran period. Initially the fecal vitamin B_6 level for subject 1 also dropped substantially, but did not change with the second bran treatment. Throughout the study, the fecal vitamin levels

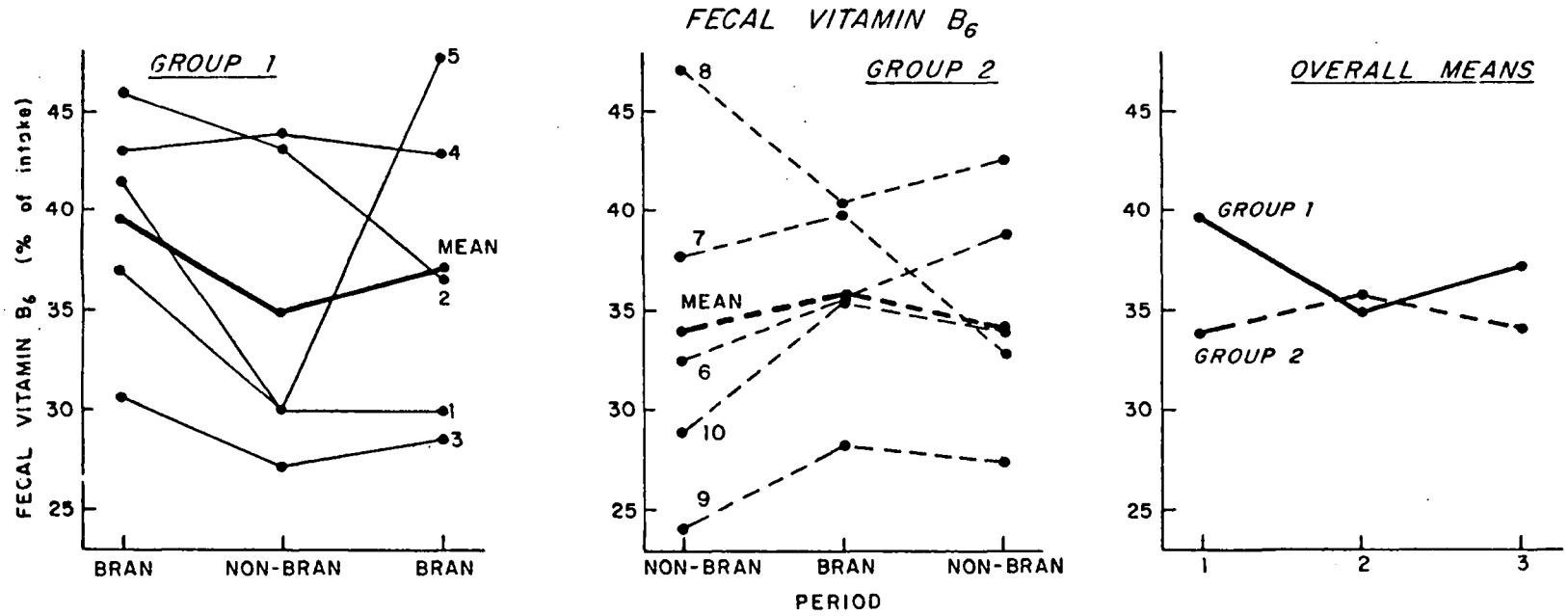

Figure 4. Fecal vitamin B_6, expressed as % of intake. Individual and group mean response to wheat bran supplementation.

Each point represents the mean of the last three 3-day composites.

cantinuously declined for subject 2 and increased for subject 6. The response of subjects 4 and 8 to bran supplementation was the inverse of the mean response of the two groups and of what one might predict from previous studies (Perera, Peffers, 1977).

Total Vitamin B_6 in Urine

For all subjects the mean total urinary vitamin B_6, expressed as % of intake was 7.7 $\pm$ 1.1% with and 7.9 $\pm$ 1.6% without wheat bran. In group 1 the subjects' mean total urinary vitamin B_6 was 8.3 $\pm$ 1.0% with bran, increased to 9.2 $\pm$ 1.5% without bran, and then decreased to 7.6 $\pm$ 0.9% with bran. The tatal vitamin B_6 in urine was the highest during the non-bran treatment in every subject in this group (see figure 5 and table 5).

The response in group 2 was much less consistent. The mean total urinary vitamin B_6 was 7.5 $\pm$ 1.4%, 7.3 $\pm$ 1.0%, and 6.9 $\pm$ 1.2% in the non-bran, bran and non-bran periods, respectively. Among the subjects in group 2, vitamin B_6 in urine was slightly higher during both of the non-bran periods than the bran period only in subject 6. During the experimental periods, the vitamin levels gradually and continuously declined in subjects 7, 8, and 10. For subject 9, urinary vitamin B_6 was slightly higher during the bran treatment than the non-bran treatments. In all of the subjects in group 1 and in three of the subjects in graup 2, urinary vitamin B_6 decreased from the last day of the adjustment period to the first period (see figure 5).

4-Pryidoxic Acid in Urine

Urinary 4-PA levels changed much more dramatically than urinary vitamin B_6 levels with wheat bran supplementation (see figure 6 and table 5). The mean urinary 4-PA, expressed as % of intake, increased for all subjects from 36.6 $\pm$ 7.5% with bran ta 42.6 $\pm$ 7.5% withaut bran. For both groups the mean urinary 4-PA was highest during the adjustment period and higher during the non-bran than the bran treatments. In group 1 the mean values were 36.8 $\pm$ 9.5%,

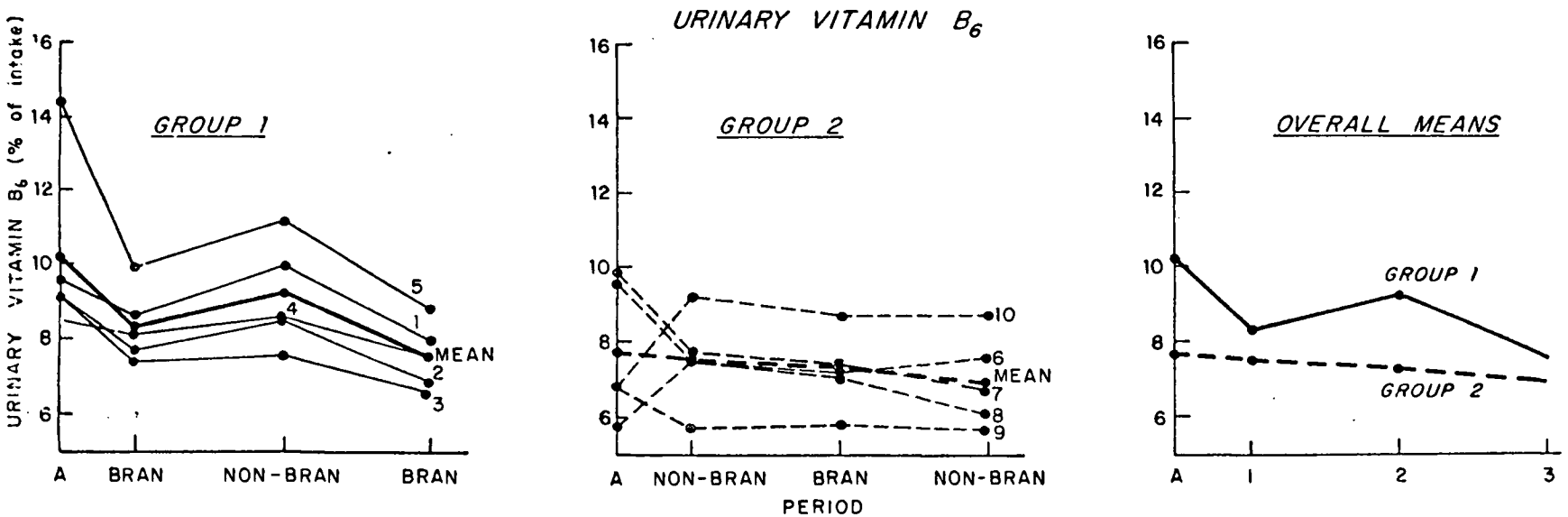

Figure 5. Urinary vitamin B_6, expressed as % af intake. Individual and averall graup mean respanse ta wheat bran supplementation.

The data paints at A represent the last day of the adjustment period. All other points represent the means of the last three values af each periad.

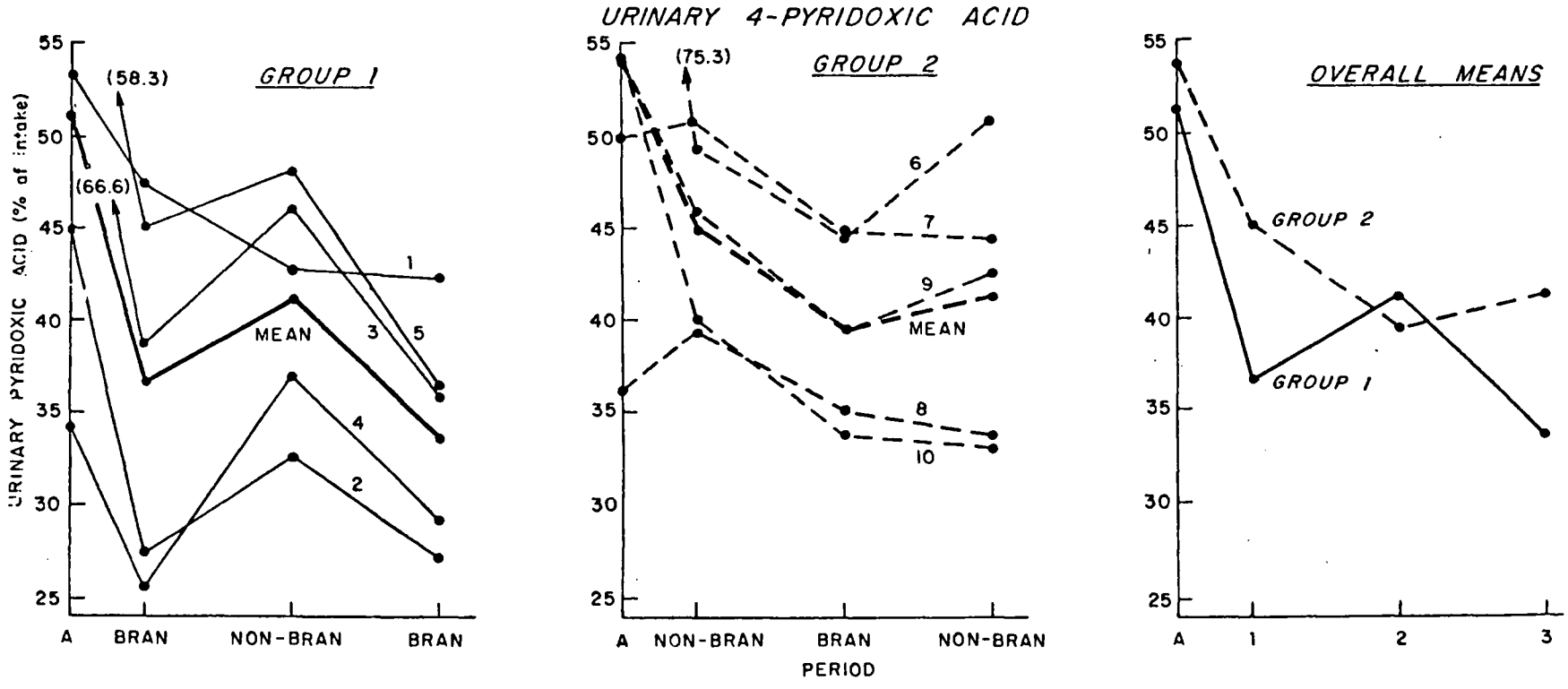

Figure 6. Urinary 4-PA, expressed as % in intake. Individual and averall group mean response ta wheat bran supplementatian.

The data points at A represent the last day of the adjustment period. All other points represent the means of the last three values of each period.

41.3 ± 8.3%, and 33.6 ± 5.6% during the first bran, the non-bran, and the second bran treatments, respectively. For subjects 2 through 5 the urinary 4-PA rose sharply when there was no bran supplement, and then fell sharply when bran was again added to the diet. For subject 1, urinary 4-PA declined throughout the experiment.

In group 2 the subjects' mean urinary 4-PA levels were 45.1 ± 5.6%, 39.4 ± 5.5%, and 41.2 ± 8.3% in the first non-bran, the bran, and the second non-bran periods, respectively. In all five subjects in this group, urinary 4-PA levels declined when the bran supplement was given. But only in subjects 6 and 9 did the levels increase when bran was deleted from the diet for the second time. In the other three subjects (7, 8, 10) urinary 4-PA continued to decline during the second non-bran period, but not as fast as during the bran period.

As illustrated in figure 7, while total fecal vitamin B_6 increased with bran, urinary 4-PA decreased in both groups. In group 1 total urinary vitamin B_6 also increased with bran. There was a significant negative correlation between fecal vitamin B_6 and urinary 4-PA ($p<0.05$, $r = -0.2702$).

Total Vitamin B_6 Excretion

The mean total excretion of vitamin B_6 and 4-PA was lower during the bran periods than the non-bran periods for group 1. Expressed as % of intake, total exretion was 84.8%, 85.4%, and 78.4% during the bran, non-bran, and bran periods respectively. In group 2 total excretion decreased from 86.6% to 82.5% with the bran treatment, and then decreased slightly to 82.3% with the second non-bran treatment (see figure 8 and table 6).

Plasma Total Vitamin B_6

The mean plasma total vitamin B_6 expressed as nmoles/dl, for all subjects was 4.54 ± 0.89 with and 4.74 ± 0.81 without supplemental wheat bran. In both groups, mean plasma vitamin B_6 levels were lowest when the subjects were receiving bran. In group 1 the mean values were 5.02 ± 0.68, 5.11 ± 0.76, and

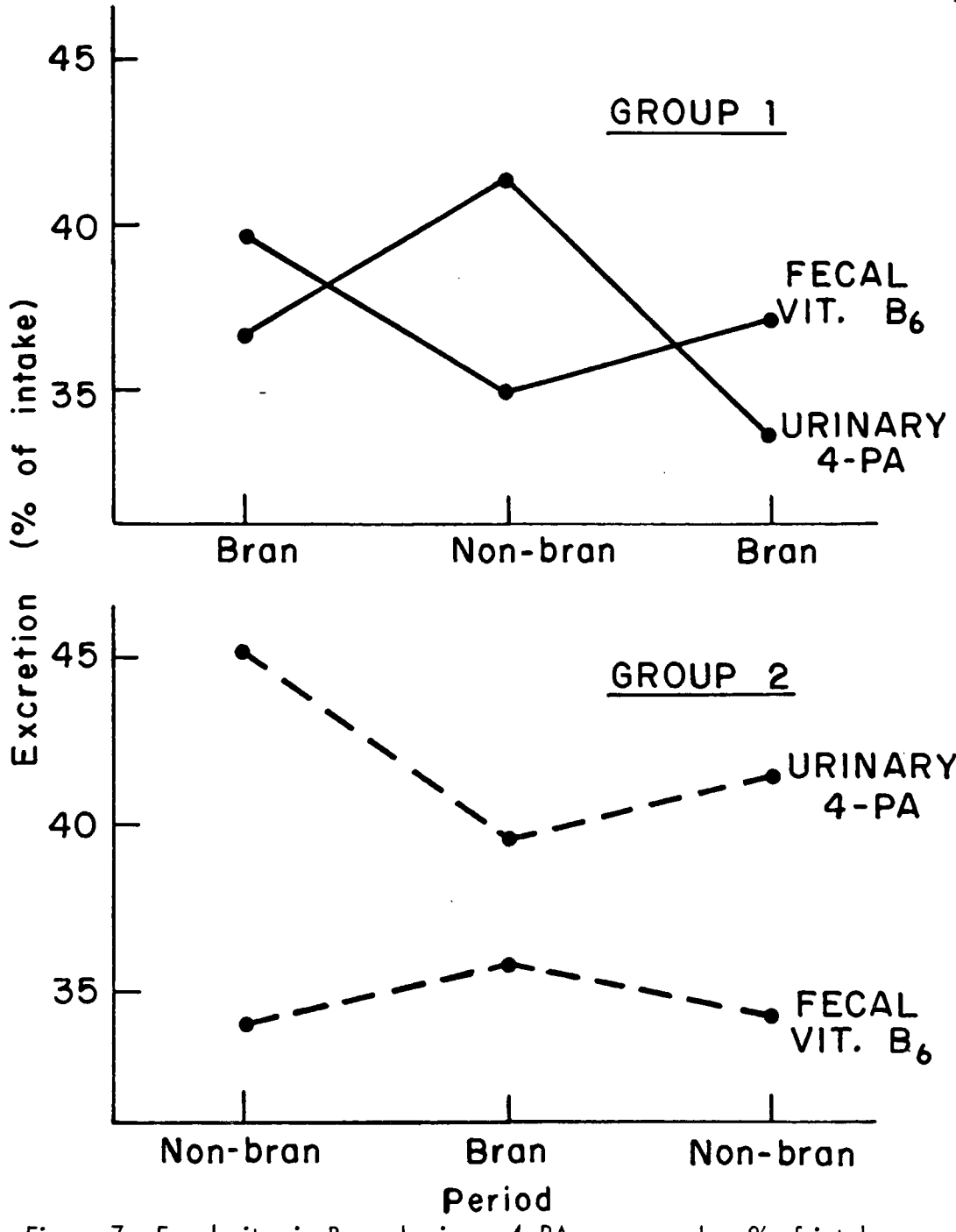

Figure 7. Fecal vitamin B_6 and urinary 4-PA, expressed as % of intake. Overall group mean response to wheat bran supplementation.

Each point represents the mean of the last three values of each period.

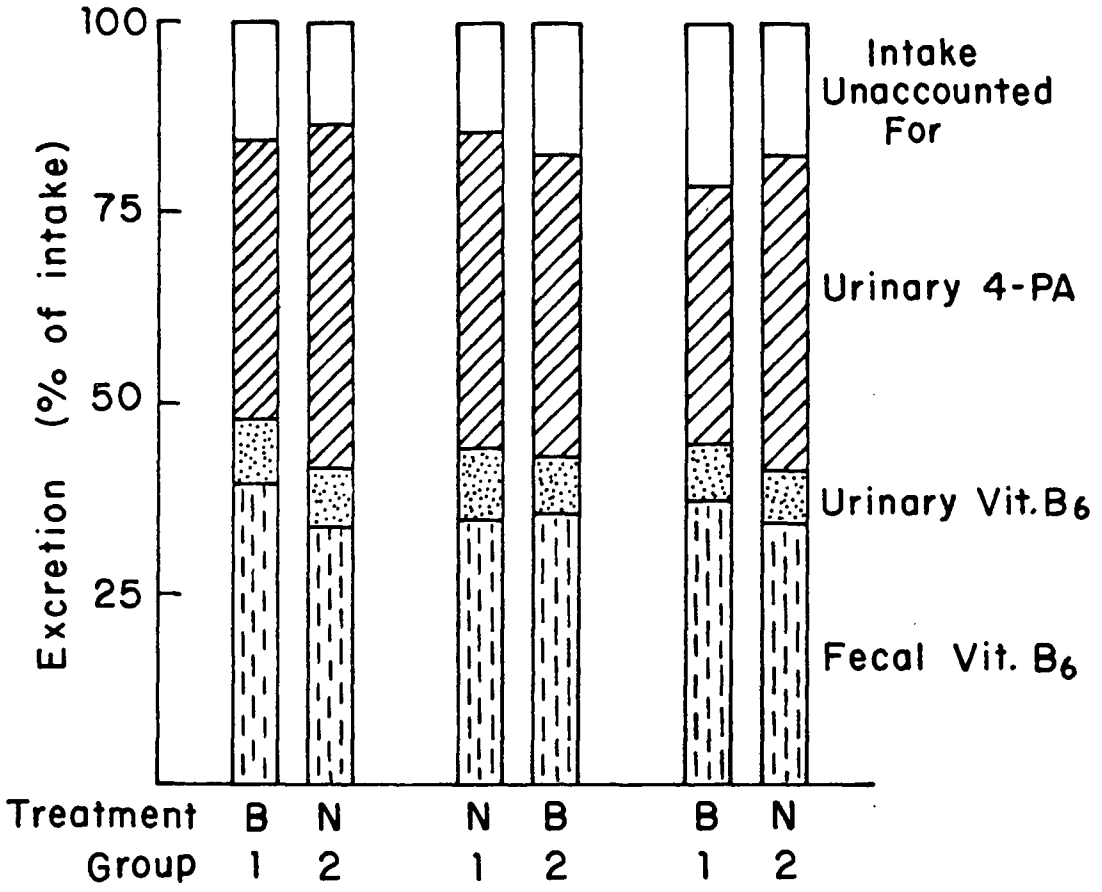

Figure 8. Total excretion of vitamin B_6 and 4-PA, expressed as % of intake.

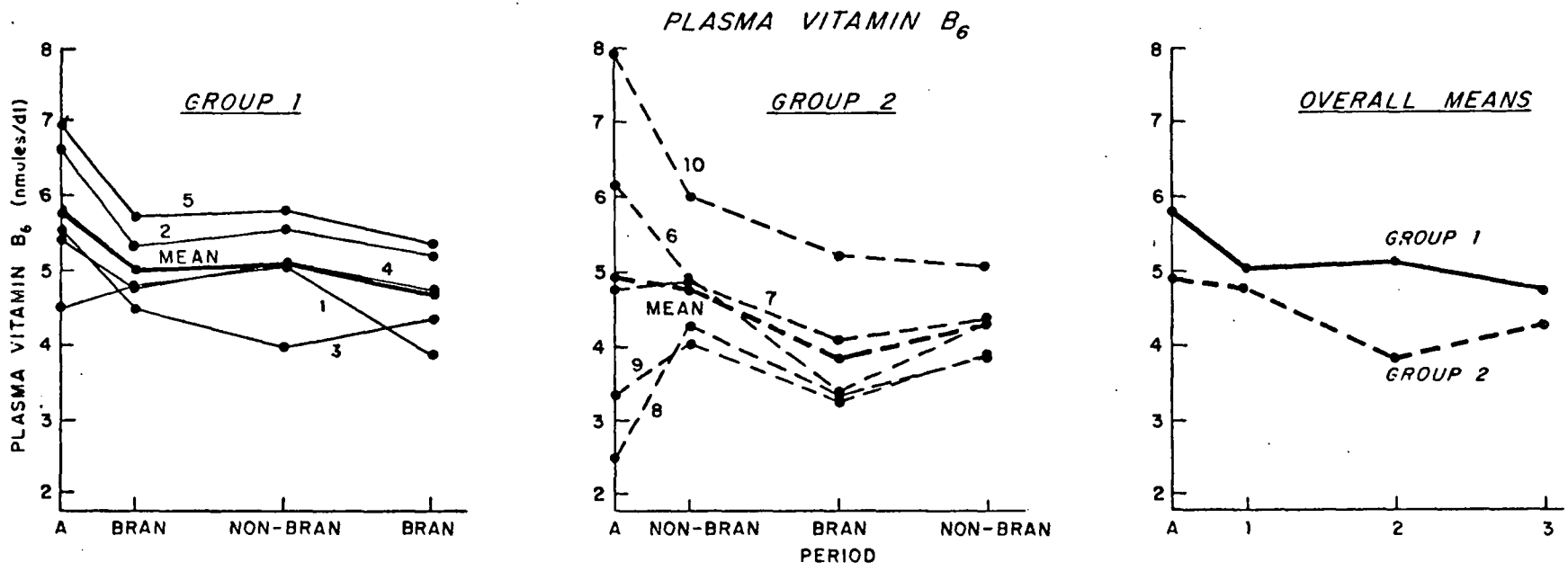

Figure 9. Plasma vitamin B_6, expressed in nmoles/dl. Individual and overall group mean response to wheat bran supplementation.

The data points at A represent the last day of the adjustment period. All other points represent the means of the last three values of each period.

4.74 ± 0.69 nmoles/dl during the first bran, the non-bran, and the second bran treatments, respectively (see figure 9 and table 5). The mean plasma total vitamin B_6 levels for subjects 1, 2, 4 and 5 were lowest during bran supplementation. In contrast, the vitamin B_6 levels for subject 3 were highest during the bran periods.

In group 2 the response to bran supplementation was more pronounced. From 4.78 ± 0.90 nmoles/dl during the first non-bran period, the subjects' mean plasma total vitamin B_6 decreased to 3.83 ± 0.79 nmoles/dl in the bran period, and increased to 4.27 ± 0.49 nmoles/dl in the second non-bran period. When bran was added to the diet, the plasma vitamin B_6 values declined in all subjects in this group. The values increased again when bran was removed, in subjects 6 through 9. For subject 10, whose initial plasma vitamin level at the end of the adjustment period was much higher than that of the other subjects, the vitamon B_6 levels continued to drop throughout the entire experiment. The rate of this decline gradually slowed as the experiment progressed.

Pyridoxal Phospate in Plasma

The mean plasma PLP, in nmoles/dl, for all subjects was 3.55 ± 0.86 with and 3.53 ± 0.89 without supplemental wheat bran. The subjects' mean plasma PLP in group 1 was 3.68 ± 0.80 nmoles/dl in the first bran period, increased to 4.33 ± 0.65 nmoles/dl in the non-bran period, and decreased to 3.97 ± 0.84 nmoles/dl in the second bran period (see figure 10 and table 5). For all subjects in this group plasma PLP levels were lower during the first bran period than in the non-bran period. The levels for subjects 1, 4, and 5 declined again during the second bran period. In these subjects the differences between treatments were distinct. In subjects 2 and 3 there were smaller differences between treatments, and the plasma PLP levels continued to rise throughout the experiment.

The subjects' plasma PLP values varied less with bran supplementation in group 2 than in group 1. In group 2 the mean plasma PLP, in nmoles/dl, was

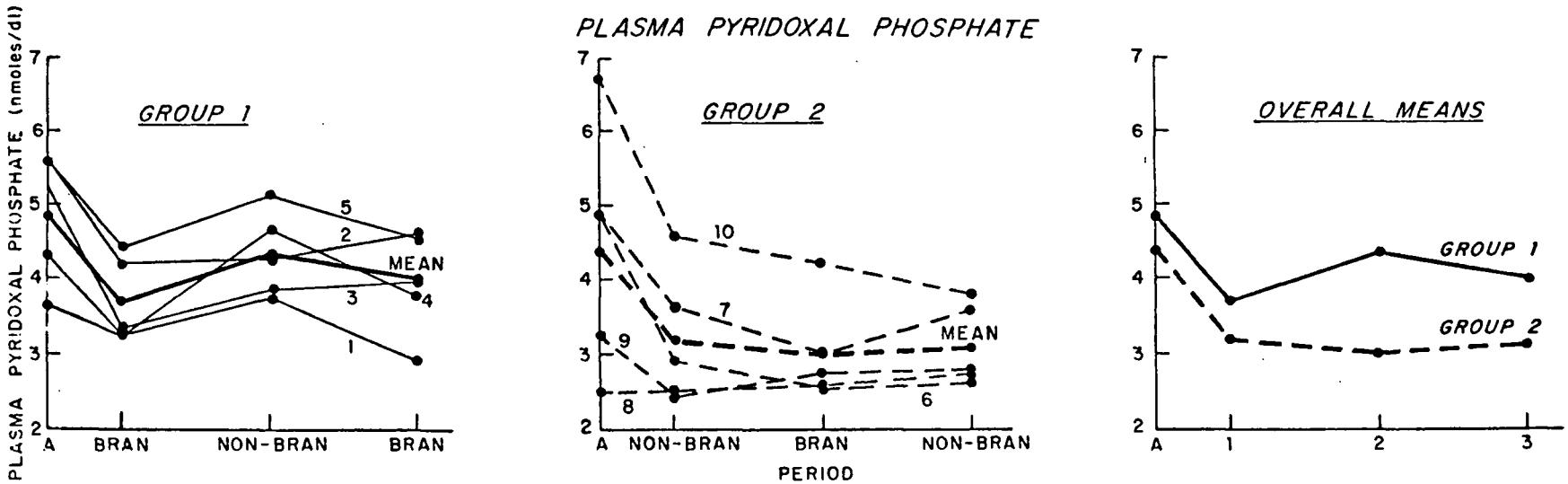

Figure 10. Plasmo PLP expressed in nmoles/dl. Individual ond overall group mean response to wheot bran supplementation.

The data points at A represent the last day of the odjustment period. All other points represent the means of the last three values of each period.

3.19 ± 0.85, 2.99 ± 0.67, and 3.08 ± 0.53 in the first non-bran, the bran, and in the second non-bran periods. The bran treatment results in a fall in plasma PLP in subjects 6, 7, and 10. However, subsequent removal of bran led to a marked increase in plasma PLP in subject 7, a slight increase in subject 6, and another decrease in subject 10. As for plasma total vitamon B_6 (figure 9 and table 5), subject 10's initial adjustment value was much higher than all of the other subjects. Although the mean plasma vitamin B_6 and PLP at the end of the adjustment period were higher in group 1 than in group 2, the range of individual values was much broader in group 2 than in group 1 (see figures 9 and 10). Throughout the experiment the subjects' mean plasma PLP ($p<0.01$), plasma total vitamin B_6 ($p<0.05$) and fecal total vitamin B_6 values were consistently higher in group 1 than in group 2.

Table 5. Individual and overall group means for each period: fecal vitamin B_6, urinary vitamin B_6 and 4-PA, plasma vitamin B_6 and PLP

FECAL VITAMIN B_6 (% of intake)

Treatment	Subject Number					Overall Mean
Group 1	(1)	(2)	(3)	(4)	(5)	
Bran	41.6 ±0.9	46.0 ± 10.2	30.7 ±4.6	43.1 ±8.8	37.0 ±9.3	39.7 ± 8.5
Non-bran	30.0 ±2.0	43.2 ± 6.1	27.2 ±0.2	44.0 ±2.4	30.1 ±7.0	34.9 ± 8.3
Bran	30.0 ±4.5	36.6 ±10.9	28.5 ±2.8	47.7 ±8.6	47.7 ±30.7	37.2 ± 15.0
Group 2	(6)	(7)	(8)	(9)	(10)	
Non-bran	32.5 ±7.1	37.7 ±6.2	47.0 ±14.3	24.1 ±5.4	28.9 ±5.2	34.0 ±10.8
Bran	35.5 ±3.5	39.8 ±9.9	40.4 ± 8.4	28.2 ±1.0	35.3 ±3.6	35.8 ± 6.9
Non-bran	38.7 ±0.7	33.0 ±1.0	42.5 ± 9.8	27.2 ±3.4	33.9 ±3.6	34.2 ± 6.5

URINARY VITAMIN B_6 (% of intake)

Group 1	(1)	(2)	(3)	(4)	(5)	
Adjustment	9.6	9.1	9.1	8.5	14.5	10.2 ±2.5
Bran	8.6 ±0.1	7.7 ±0.7	7.4 ±0.5	8.1 ±0.4	9.9 ±0.7	8.3 ±1.0
Non-bran	10.0 ±0.5	8.5 ±0.4	7.6 ±0.3	8.6 ±0.2	11.2 ±1.9	9.2 ±1.5
Bran	8.0 ±0.7	6.9 ±0.7	6.6 ±4.5	7.6 ±5.6	8.8 ±4.0	7.6 ±0.9
Group 2	(6)	(7)	(8)	(9)	(10)	
Adjustment	9.6	9.9	5.7	6.8	6.8	7.8 ±1.9
Non-bran	7.5 ±0.3	7.7 ±0.5	7.5 ±0.6	5.7 ±0.1	9.2 ±1.8	7.5 ±1.4
Bran	7.2 ±0.3	7.4 ±0.3	7.1 ±0.7	5.8 ±0.3	8.7 ±0.3	7.3 ±1.0
Non-bran	7.7 ±0.3	6.7 ±0.9	6.1 ±0.8	5.7 ±0.2	8.7 ±0.1	6.9 ±1.2

Table 5. (Continued)

URINARY 4-PYRIDOXIC ACID

Treatment	Subject Number					Overall Mean
Group 1	(1)	(2)	(3)	(4)	(5)	
Adjustment	53.7	45.1	58.3	21.1	66.6	49.0 ±17.4
Bran	47.4 ±2.6	27.4 ±2.7	38.7 ±1.3	25.5 ±2.1	45.0 ±4.0	36.8 ± 9.5
Non-bran	42.8 ±8.5	32.6 ±5.1	46.1 ±2.0	36.9 ±2.7	48.2 ±11.0	41.3 ± 8.3
Bran	42.3 ±1.9	27.2 ±0.2	35.9 ±2.5	29.2 ±1.2	36.5 ± 3.7	33.6 ± 5.6
Group 2	(6)	(7)	(8)	(9)	(10)	
Adjustment	75.3	49.9	36.0	54.2	54.2	53.9 ±14.1
Non-bran	49.3 ±3.8	50.7 ±4.0	39.3 ±4.0	45.9 ±1.5	40.0 ±2.8	45.1 ± 5.6
Bran	44.3 ±1.5	44.7 ±3.2	34.9 ± 4.2	39.3 ± 2.6	33.6 ± 3.3	39.4 ± 5.5
Non-bran	50.6 ±4.7	44.2 ±8.1	33.6 ±4.6	42.3 ±3.1	32.9 ±9.4	41.2 ± 8.3

PLASMA VITAMIN B_6 (nmole/dl)

Group 1	(1)	(2)	(3)	(4)	(5)	
Adjustment	4.49	6.62	5.56	5.44	6.97	5.82 ±0.99
Bran	4.81 ±0.24	5.32 ±0.47	4.49 ±0.66	4.77 ±0.48	5.71 ±1.07	5.02 ±0.68
Non-bran	5.08 ±0.20	5.56 ±0.61	3.98 ±0.56	5.12 ±0.34	5.83 ±0.68	5.11 ±0.76
Bran	3.86 ±0.07	5.24 ±0.34	4.37 ±0.12	4.77 ±0.25	5.40 ±1.01	4.73 ± 0.69
Group 2	(6)	(7)	(8)	(9)	(10)	
Adjustment	6.15	4.73	2.48	3.31	7.92	4.92 ±2.18
Non-bran	4.90 ±0.77	4.85 ± 0.35	4.26 ±0.94	4.02 ± 0.74	5.99 ±0.25	4.78 ±0.90
Bran	3.35 ±0.27	4.06 ± 0.25	3.31 ±0.12	3.23 ±0.18	5.20 ±0.21	3.83 ±0.79
Non-Bran	4.29 ±0.29	4.33 ±0.14	3.84 ±0.25	3.86 ±0.25	5.04 ±0.25	4.27 ±0.49

Table 5. (Continued)

PLASMA PLP (nmoles/dl)

Treatment	Subject Number					Overall Mean
Group 1	(1)	(2)	(3)	(4)	(5)	
Adjustment	3.65	5.61	5.22	4.31	5.58	4.87 ±0.86
Bran	3.24 ±0.41	4.19 ±0.55	3.34 ±0.40	3.23 ±0.11	4.41 ±1.37	3.68 ±0.80
Non-Bran	3.74 ±0.33	4.27 ±0.52	3.85 ±0.34	4.67 ±0.03	5.12 ±0.71	4.33 ±0.65
Bran	2.91 ±0.27	4.63 ±0.51	3.97 ±0.40	3.77 ±0.12	4.56 ±1.20	3.97 ±0.84
Group 2	(6)	(7)	(8)	(9)	(10)	
Adjustment	4.84	4.88	2.48	3.22	6.70	4.42 ± 1.64
Non-bran	2.89 ±0.19	3.61 ±0.22	2.49 ±0.10	2.42 ±0.07	4.57 ±0.35	3.19 ±0.85
Bran	2.52 ±0.20	2.99 ±0.12	2.56 ±0.13	2.72 ±0.20	4.19 ±0.37	2.99 ± 0.67
Non-bran	2.59 ±0.23	3.57 ± 0.35	2.71 ±0.26	2.77 ±0.19	3.77 ± 0.26	3.08 ±0.53

Table 6. Individual and overall group means for each period: total excretion of fecal and urinary vitamin B_6 and urinary 4-PA.

Group I

	(1)	(2)	(3)	(4)	(5)	Overall mean
Bran						
Fecal vitamin B_6	41.6 ±0.9	46.0 ± 10.2	30.7 ±4.6	43.1 ±8.8	37.0 ±9.3	39.7 ±8.5
Urine vitamin B_6	8.6 ±0.1	7.7 ±0.7	7.4 ±0.5	8.1 ±0.4	9.9 ±0.7	8.3 ±1.0
Urine 4-PA	47.4 ±2.6	27.4 ±2.7	38.7 ±1.3	25.5 ±2.1	45.0 ±4.0	36.8 ±9.5
Total	97.6	81.1	76.8	76.7	91.9	84.8
Non-Bran						
Fecal vitamin B_6	30.0 ±2.0	43.2 ±6.1	27.2 ±0.2	44.0 ±2.4	30.1 ±7.0	34.9 ±8.3
Urine vitamin B_6	10.0 ±0.5	8.5 ±0.4	7.6 ±0.3	8.6 ± 0.2	11.2 ±1.9	9.2 ±1.5
Urine 4-PA	42.8 ±8.5	32.6 ±5.1	46.1 ±2.0	36.9 ± 2.7	48.2 ±11.0	41.3 ±8.3
Total	82.8	84.3	80.9	89.5	89.5	85.4
Bran						
Fecal vitamin B_6	30.0 ±4.5	36.6 ± 10.9	28.5 ±2.8	43.0 ±8.6	47.7 ±30.7	37.2 ±15.0
Urine vitamin B_6	8.0 ±0.7	6.9 ±0.7	6.6 ±4.5	7.6 ±5.6	8.8 ±4.0	7.6 ±0.9
Urine 4-PA	42.3 ±1.9	27.2 ±0.2	35.9 ± 2.5	29.2 ±1.2	36.5 ±3.7	33.6 ±5.6
Total	80.3	70.7	71.0	79.8	93.0	78.4

Table 6. (Continued)

Group 2

Non-Bran	(6)	(7)	(8)	(9)	(10)	Overall mean
Fecal vitamin B_6	32.5 ± 7.1	37.7 ± 6.2	47.0 ± 14.3	24.1 ± 5.4	28.9 ± 5.2	34.0 ± 10.8
Urine vitamin B_6	7.5 ± 0.3	7.7 ± 0.5	7.5 ± 0.6	5.7 ± 0.1	9.2 ± 1.8	7.5 ± 1.4
Urine 4-PA	49.3 ± 3.8	50.7 ± 4.0	39.3 ± 4.0	45.9 ± 1.5	40.0 ± 2.8	45.1 ± 5.6
Total	89.3	96.1	93.8	75.7	78.1	86.6
Bran						
Fecal vitamin B_6	35.5 ± 3.5	39.8 ± 9.9	40.4 ± 8.4	28.2 ± 1.0	35.3 ± 3.6	35.8 ± 6.9
Urine vitamin B_6	7.2 ± 0.3	7.4 ± 0.3	7.1 ± 0.7	5.8 ± 0 .3	8.7 ± 0.3	7.3 ± 1.0
Urine 4-PA	44.3 ± 1.5	44.7 ± 3.2	34.9 ± 4.2	39.3 ± 2.6	33.6 ± 3.3	39.4 ± 5.5
Total	87.0	91.9	82.4	73.3	77.6	82.5
Non-Bran						
Fecal vitamin B_6	38.7 ± 0.7	33.0 ± 1.0	42.5 ± 9.8	27.2 ± 3.4	33.9 ± 3.6	34.2 ± 6.5
Urine vitamin B_6	7.7 ± 0.3	6.7 ± 0.9	6.1 ± 0.8	5.7 ± 0.2	8.7 ± 0.1	6.9 ± 1.2
Urine 4-PA	50.6 ± 4.7	44.2 ± 8.1	33.6 ± 4.6	42.3 ± 3.1	32.0 ± 9.4	41.2 ± 8.3
Total	97.0	83.9	82.2	75.2	75.5	82.3

DISCUSSION

Available is defined as "capable of being used advantageously; usable; at one's disposal" (Funk, 1956). Expanding from this, bioavailability of a specific nutrient can be defined as the extent to which that nutrient is capable of being used advantageously by the body. The nutrients in the food we eat are at our disposal only if they are digested in and absorbed from our gastrointestinal tract. Bioavailability reflects the complex processes of both digestion and absorption. In turn, bioavailability of an ingested nutrient is reflected in its levels in body tissues, fluids, and excretory products. A decrease in bioavailability leads to greater excretion of that nutrient in the feces, lower excretion of it and its metabolites in the urine, and lower levels of its forms in plasma.

The various constituents in our food can interact within the intestine to influence absorption and bioavailability. Wheat bran appears to interact with other nutrients (Cummings et al. 1976 ; Eastwood and Mitchell, 1976). A recent study examined the effects of three different types of bread on the bioavailability of vitamin B_6 (Perera, 1977; Peffers, 1977). While vitamin B_6 intake was held constant, subjects consumed whole wheat bread, white bread with added vitamin B_6, or white bread with an oral vitamin B_6 supplement. During the whole wheat periods the subjects' fecal vitamin B_6 was significantly higher, and urinary 4-PA and plasma PLP were lower than during the white bread periods. These results suggest that a component of whole wheat bread may interact with the vitamin to decrease its bioavailability.

Bran is one of the components of wheat removed during the milling of white flour. As an extension of the bread study, the present experiment explored the effects of wheat bran on the bioavailability of vitamin B_6. In both individual and group data there were trends for all parameters that are consistent with the results of the above studies and the hypothesis that wheat bran reduces the bioavailability of vitamin B_6.

The determination of fecal excretion of vitamin B_6 is pivotal in the determination of bran's effect on the bioavailability of the vitamin. If bran does

interact with vitamin B_6 to decrease its absorption, it would then directly affect fecal excretion. Plasma and urinary levels are only indirectly affected. These parameters are also influenced by the body's metabolic balances and activities and normal nutrient turnover. In the present study fecal excretion of the vitamin increased in 6 subjects when bran was introduced and decreased in 7 subjects when bran was removed from the diet.

There are three possible sources for this increase in fecal vitamin B_6: 1) endogenous, from desquamated intestinal epithelial cells or intestinal secretions; 2) microbial, sythesized by the micraflora of the intestine; and 3) exogenous, fram nutrients ingested but not absarbed. The endogenous contribution to fecal vitamin B_6 is probably negligible and constant over time. The microbial origin of vitamin B_6 was first suggested as a result of experiments with animals. Early studies showed that rats consuming a vitamin B_6-deficient diet grew faster when the diet contained a complex rather than a simple carbohydrate source (Guerrant, Dutcher, and Tomey, 1935). The growth rate of vitamin B_6-deficient rats increased when they consumed the feces of other rats who were fed a vitamin B_6-deficient diet containing dextrinized corn starch. Undigested dextrins were faund in the feces of the latter group of animals. The authors suggest that growth was enhanced by the consumption of fecal vitamin B_6 formed by yeast cells found in large numbers in the colon of the aminals. The yeast utilize the camplex carbohydrate as an energy source. Similar results were found in a study by Sarma, Snell, and Elvehjem (1946).

According to a study with 6 human subjects (Fuchs, Dorfman, and Floch, 1976) anaerobic bacterial excretion significantly increased when 3 oz. af processed wheat bran was added to a regular diet. The aerobic bacterial excretion was stable. Escherichia coli, a facultative anaerabe in the human intestinal tract (Attebery, Sutter, and Finegald, 1972), sythesizes PN from glyceral, pyruvate, serine, and glucose and excretes it into the culture medium (Hill, et al. 1972; Gaodwin, T. W., 1963).

The micraflora af the human intestinal tract reside principally in the colon

(Ganong, 1969), while absorption of vitamin B_6 occurs almost entirely in the jejunum (Brain and Booth, 1964). Therefore, if vitamin B_6 is sythesized by microorganisms in the human colon, it is probably neither absorbed nor available for use in metabolism. If the vitamin B_6 of microbiological origin were available to humans, deficiency of the vitamin could not be so readily induced experimentally. After only 5 days,urine vitamin B_6 and 4-PA levels began to decline in subjects consuming 0.16 mg vitamin B_6/ day (Linkswiler, 1967).

If the vitamin B_6 sythesized by microorganisms were absorbed, urinary and plasma values would also increase with wheat bran supplementation. But in the present study the overall trends were in the opposite direction for almost all of these parameters. Urinary 4-PA was markedly higher during the non-bran than the bran periods for both groups of subjects (figure 6). And, there was a significant negative correlation ($r= -0.2702, p<0.05$) between fecal vitamin B_6 and urinary 4-PA (see figure 7). In group 1, urinary vitamin B_6 levels were somewhat lower during the bran treatments. Plasma PLP and total vitamin B_6 levels for both groups decreases slightly with bran supplementation. These results suggest that the observed increase in fecal vitamin B_6 during consumption of wheat bran is from an unavailable source and not solely of microbial origin.

During bran supplementation the decrease in urinary 4-PA was greater than the increase in fecal vitamin B_6. These two compounds are the primary excretory products of vitamin B_6. Therefore total urinary and fecal excretion of vitamin B_6 compounds was slightly lower with than without bran supplementation. This is not the predicted result if microbial synthesis of vitamin B_6 is significantly enhanced by wheat bran.

As mentioned earlier, exogenous sources of the vitamin may also contribute to the increase in fecal vitamin B_6 levels. If intestinal transit time is shortened to the extent that the intestinal contents have less contact with absorptive surfaces, nutrient absorption may decrease. Other studies have shown that wheat bran consistently decreases intestinal transit time in subjects with initially long transit time. (Payler, 1973; Cummings et al. 1976). However, in the present

study, transit time was neither significantly nor consistently altered by the bran treatment (Yu, 1979).

Studies by other researchers suggest that bran decreases absorption of some nutrients by acting as a polyfunctional cation exchanger (Cummings et al. 1976; Eastwood and Mitchell, 1976; Reinhold et al. 1975; Ismail-Beigi et al 1977). Within the small intestine the mixture of bile and pancreatic secretions has a pH range from 7.8 to 8.3 (Guyton, 1976). In this pH range, the amino group of PM is primarily in the protonated, positively charged, form.

The amount of wheat bran administered in this study provided 0.186 mg or 1.10 μmoles vitamin B_6/day. Of this 36%, or 0.40 μmoles may be bound in wheat bran (Siegel, Melnick, and Oser, 1943). If all of the bound vitamin is excreted with the undigestible components of bran, one would expect an increase of at least 0.40 μmoles vitamin B_6day in the feces during bran supplementation. For individual subjects the difference between fecal vitamin B_6 excretion during a bran and an adjacent non-bran period ranged from -0.58 to 1.82 μmoles/day. This difference was greater than or equal to 0.40 μmoles/day in 7 out of 20 possible instances, between 0 and 0.40 μmoles/day in 10 cases, and less than 0 in 3 cases. The 13 values less than 0.40 suggest that at least some of the bound vitamin is available for absorption in the jejunum or for metabolism by microorganisms. The higher values indicate that the increase in fecal vitamin B_6 was not solely due to the bound vitamin in wheat bran. In only two instances was the increase in fecal vitamin B_6 with bran supplementation greater than the total amount of vitamin B_6 in 15 g of bran. From these results it is difficult to determine if fecal vitamin B_6 increased because the vitamin in bran is unavailable or because wheat bran decreases the availability of the vitamin from other foods consumed with bran.

If vitamin B_6 absorption decreases during constant consumption of the vitamin, urinary vitamin B_6 and 4-PA should also decrease. Although urinary exretion of the vitamin and its metabolite, 4-PA, is not solely a function of vitamin B_6 intake, it does decline during vitamin B_6 depletion (Linkswiler, 1967;

Baker, et al. 1964; Baysal et al. 1966). Linkswiler (1967) found that urinary 4-PA levels decreased more rapidly during depletion than the vitamin B_6 levels. In the study by Perera (1977) urinary 4-PA values were lower with whole wheat than either of two white breads. However, urinary vitamin B_6 values were not significantly different. In the present experiment urinary 4-PA also decreased more rapidly and more consistently with bran supplementation than urinary vitamin B_6 (figures 5 and 6).

Plasma vitamin B_6 and PLP should also decline when absorption decreases. In a study substantiating the role of plasma PLP as an indicator of both vitamin B_6 nutriture and body stores, plasma PLP in rats increased as PN intake ranged from 0 to 100 μg/day (Lumeng and Li, 1978). Studies with humans have shown that plasma PLP values remain remarkably constant over time (Lumeng and Li, 1974). This may be partly explained by the fact that plasma PLP is in dynamic equilibrium with hepatic syntheses and cellular degradation or extraction (Lumeng et al. 1974). Peffers (1977) found that both plasma PLP and vitamin B_6 concentrations were lower when subjects consumed whole wheat bread than either of the two types of white bread. In the present study these two plasma parameters were lower during the bran than the non-bran treatments. However, the change with bran supplementation was slight (figures 9 and 10).

In spite of the consistent trends in the data, there were no statistically significant differences between the bran and the non-bran treatments for any of the parameters studied. Various factors may account for this. The sample size was small and the variation among and within individuals was large, especially in fecal vitamin B_6 and urinary 4-PA. The variation in fecal values has been noted by other authors (Allen, Raynolds, and Margen, 1979).

There was a difference between the two groups' adjustment values and their subsequent response to bran supplementation. Urinary and plasma vitamin B_6 ($p<0.05$), and plasma PLP ($p<0.01$) values showed a statistically significant difference between the two groups that was not related to treatment. Except for urinary 4-PA, group 1 had consistently higher values than group 2 throughout

The adjustment and experimental periods. There was a more consistent and pronounced change in fecal and urinary vitamin B_6, urinary 4-PA, and plasma PLP levels with bran supplementation in group 1 than in group 2 (table 5). From the adjustment to the first period, the mean urinary vitamin B_6, 4-PA, and plasma vitamin B_6 values dropped more sharply in group 1 than in group 2. This is the expected response since group 1 received the bran supplement first.

The mean blood and urine values for both groups were considerably higher at the end of the adjustment period than at any other time during the study. The range of these values was wide during the adjustment period and gradually narrowed as the experiment progressed. These data suggest that prior to the experiment the subjects were consuming more vitamin B_6/day than during the study. Subject 10, whose initial plasma PLP and vitamin B_6 values were much greater than the other subjects, was taking a vitamin supplement containing 2.0 mg vitamin B_6 daily for 6 months before the experiment began. He appeared to require a longer period of time to adjust to the lower vitamin B_6 intake than the other subjects.

Students t test showed a significant difference between urinary vitamin B_6 levels during the first and second bran treatments for group 1 ($p<0.05$), and between urinary 4-PA levels during the first and second non-bran treatments for groups 2 ($p\leq0.05$). The mean fecal, urinary, and plasma vitamin B_6 and the mean urinary 4-PA values for group 1 were higher during the first than the second bran treatments. The mean urinary and plasma vitamin B_6, urinary 4-PA, and plasma PLP values for group 2 were higher during the first than the second non-bran treatment (figures 4 through 10). High values during the first period probably reflect the subjects' high pre-experimental intake of vitamin B_6. The initial adjustment period in this study was brief. A longer adjustment period may be necessary to minimize the influence of pre-experimental diets on experimental data.

A recent study by Allen, et al. (1979) substantiates the hypothesis that, especially for fecal data, a 4-day adjustment period is not long enough. A

water saluble fecal marker, polyethylene glycal (PEG) was fed daily and measured quantitatively in the feces af subject receiving liquid formula diets. It was assumed that all of the pre-experimental diet had been excreted when PEG/g dry fecal salids became constant. By this criterion, it taak 8 - 10 days far 80% af the subjects to excrete all af the pre-experimental diet. When PEG was fed anly ance it taak mare than 9 days for 9 aut af 20 subjects ta excrete all of the ingested PEG. This pracess taak mare than 12 days in ane subject. The authars nate that some subjects appear ta paal their intestinal contents. In the present experiment, data fram the last 9 days af each periad were averaged providing in essence anather 9 days of adjustment ta each new treatment. Hawever, the evidence from Allen and cowarkers, and the higher values during period 1 than period 3 in both graups suggest that even this may nat have been an adequate adjustment periad far some subjects.

The amaunt af wheat bran administered in this study, 15 g, may nat have been sufficient ta effect a significant change in the excretian and plasma levels af vitamin B_6. This quantity af raw bran contains anly 7.1 g af neutral detergent fiber (Yu, 1979). Fecal excretian af selected cations and anians increased with increased dietary fiber in a study by Cummings, et al. (1976). The amaunt of fiber added ta the diet (28 g) was greater and the duratian of the cantral and experimental periods (3 weeks each) was longer than in the present experiment.

Hawever, anly 16 g af raw bran significantly increased fecal excretian of Na, K, and Mg in a study by Eastwood and Mitchell (1976). Althaugh we administered almost that amaunt of raw bran, the bran was baked in the breakfast cereal, muffins, and meat befare it was cansumed. Caaking may diminish same af the effects af wheat bran. Raw bran was cansistently mare effective than caoked bran of equivalent crude fiber cantent in decreasing transit time, increasing fecal dry weight, and increasing stool volume (Wyman et al. 1976). Neither 13.2 nor 22 g af caoked bran/day significantly altered any of the abave parameters.

Urinary and plasma vitamin B_6 values change significantly during depletian

and repletion (Linkswiler, 1967; Baysal et al. 1966). For discussion purposes, "effective" intake may be defined as dietary intake minus fecal excretion. The change in "effective" vitamin B_6 intake, and therefore also urinary excretion and plasma values, is much more subtle in this experiment with wheat bran than in the depletion studies. Consumption of more than 15 g of wheat bran may be necessary to decrease "effective" intake, and thus significantly decrease blood and urine values.

Conclusions

Although there were no statistically significant differences between the bran and non-bran treatments, there were consistent trends in the data suggesting that wheat bran decreases the bioavailability of vitamin B_6. While fecal vitamin B_6 increased, the urinary and plasma values generally declined with wheat bran supplementation. Since there was a statistically significant negative correlation ($p<0.05$, $r = -0.2702$) between fecal vitamin B_6 and urinary 4-PA, the increase in fecal vitamin levels probably reflect a vitamin source which is not available for absorption and metabolism. The difference between fecal vitamin B_6 with and without bran was greater than the amount of the vitamin in 15 g of wheat bran only in 2 out of 20 possible instances when bran was either added to or removed from the diet. Therefore, it is impossible to determine if only the vitamin B_6 in bran is unavailable, or if bran decreases the availability of vitamin B_6 in other foods. Further research is needed to clarify this point.

The results of this study suggest that 15 g of wheat bran does not adversely affect vitamin B_6 status when intake of the vitamin is adequate. The long-term effects of bran consumption cannot be determined, however, from this short-term experiment. If one's vitamin B_6 status is initially poor, and aggravated by oral contraceptives (Miller, Dow, and Kokkeler, 1978) or alcohol (Lumeng and Li, 1974), a high intake of wheat bran over an extended period of time may further deprive the body of necessary vitamin B_6. A well-balanced diet which includes fresh fruits and vegetables, legumes, meat, fish, poultry, milk products and

whole grains provides adequate amounts of both vitamin B_6 and fiber. Moderate amounts of wheat bran probably can be added to this type of diet without jeopardizing vitamin B_6 status.

Suggestions for Further Study

More conclusive results might be obtained if some of the following alterations in experimental design were made. Since the subjects tolerated well the 15 g of wheat bran, more bran could be administered. The subjects should receive each treatment for a longer period of time. A control period could be followed by a bran and a non-bran period. PEG could be administered once at the beginning of each period. After all the PEG is excreted, and therefore all of the pre-period diet, data would be collected for 2 weeks for each period. At the end of the control period the subjects could be matched according to their values for the parameters to be studied and divided into two groups. The groups could receive the bran and the non-bran diets in opposite order.

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APPENDIX

Appendix Table 1. Analytical data for AACC Certified Food Grade Wheat Bran RO7-3691 (AACC, 1976)

Assay	%	Assay	mg/100g	Assay	ppm
Crude fiber	8.91	Water holding capacity	9500	Aluminum	5.0
Protein	14.3	Thiamine	0.78	Arsenic	<0.1
Moisture	10.4	Riboflavin	0.39	Barium	45.07
Fat (acid hydrolysis)	5.22	Niacin	20.9	Boron	4.5
Ash	5.12	Pyridoxine	0.58	Cadmium	2.8
Acid detergent fiber	11.9	Folic acid	0.12	Cobalt	39.2
Neutral detergent fiber	40.2	Pantothenic acid	2.48	Copper	15.6
Lignin	3.2	Vitamin E	2.69	Iron	122
Pectin	3.0	Choline	228.0	Lead	2.3
Calcium	0.12	B-sitisterol	123	Manganese	80.0
Magnesium	0.43	Campesterol	68.8	Mercury	0.002
Phosphorus	1.04	Stigmasterol	11.2	Selenium	0.1
Potassium	1.38			Zinc	54.5
Sodium	0.10			Aflatoxin	<0.00001
Damaged starch	3.74			Pesticides, phosphorus containing	<0.005
Total starch	17.4				
Total sugar as invert	7.04			Pesticides, chlorine containig	<0.02
Pentosan	22.1				
Phytic acid	3.36				

Particle Size	%
On US# 10	1
12	2
14	5
16	11
18	13
20	9

Particle Size	%
On US# 30	33
40	17
50	8
60	1
70	trace

Appendix Table 2. Results of the serum chemical screen[a], performed at beginning and end of the experiment.

Serum values	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Triglycerides	140[b]	100	52	139	116	126	74	144	137	82
(mg/dl)	70[c]	176	58	116	97	70	43	97	85	54
Lactate dehydrogenase	100	119	98	103	88	112	72	82	96	68
(u/liter)	70	94	103	133	83	83	60	87	87	83
Glutamate oxalo acetate	27	34	-	31	22	14	16	19	25	14
transaminase (u/liter)	20	34	33	27	20	18	16	25	18	25
Glutamate pyruvate	92	127	33	47	44	47	14	18	41	18
Transaminase (u/liter)	16	119	22	57	29	16	8	16	16	37
Phosphorus	3.8	3.7	2.8	3.8	4.7	4.0	3.7	3.3	3.7	4.3
(mg/dl)	3.7	3.3	3.4	2.9	3.2	4.4	3.9	3.3	3.2	3.6
Calcium	10.1	9.8	9.2	10.0	10.5	10.2	9.1	9.7	10.1	9.5
(mg/dl)	9.8	9.0	9.4	9.0	9.4	9.7	9.1	9.2	9.8	9.5
Alkaline phosphatase	18	25	10	22	26	22	18	16	18	21
(u/liter)	15	24	12	18	25	23	18	16	16	19
Bilirubin	2.0	0.7	0.7	1.1	0.6	0.8	0.8	0.8	1.0	0.7
(mg/dl)	1.7	0.5	0.8	1.2	0.7	0.7	0.5	0.7	0.8	1.0
Uric acid	3.7	5.0	4.3	4.2	5.7	4.1	3.8	5.7	4.5	4.0
(mg/dl)	4.1	4.4	4.9	3.8	4.9	3.9	4.3	5.0	4.0	3.8
Total Protein	7.5	7.1	6.7	7.9	7.4	7.9	7.2	6.8	7.5	6.9
(g/dl)	6.4	6.4	7.0	6.4	7.0	7.6	-	6.4	6.8	7.0
Albumin	5.0	4.9	5.4	4.8	4.8	4.9	5.2	4.6	5.3	4.2
(g/dl)	5.1	4.9	5.4	4.8	4.9	5.5	-	4.9	5.0	5.2
Cholesterol	155	211	138	188	165	192	141	188	223	160
(mg/dl)	127	225	131	164	144	148	115	144	193	144
Urea nitrogen	17	14	11	12	18	12	12	16	19	14
(mg/dl)	16	14	14	11	12	12	15	11	18	12

Table 2. (Continued)

	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Glucose	145	97	83	92	103	113	107	99	85	91
(mg/dl)	92	86	93	85	99	98	102	104	86	87
Chlorine	105	101	96	105	106	100	99	98	100	103
(meq/liter)	104	104	102	103	107	103	104	104	103	103
Sodium	146	148	135	149	139	131	141	133	134	137
(meq/liter)	142	143	143	141	142	142	143	143	140	139
Potassium	4.2	4.5	3.7	4.3	4.8	5.1	4.1	4.9	4.7	4.5
(meq/liter)	4.2	4.6	4.1	4.0	4.6	4.6	5.3	5.5	5.2	4.4
Globulin	2.5	2.2	1.3	2.9	2.6	3.0	2.0	2.2	2.2	2.7
	1.3	1.5	1.6	1.6	2.1	2.1	-	1.5	1.8	1.8
Albumin/globulin	2.0	2.2	4.2	1.7	1.8	1.6	2.6	2.1	2.4	1.6
	3.9	3.3	3.4	3.0	2.3	2.6	-	3.3	2.8	2.9

a. Performed at Good Samaritan Hospital, 3600 N. W. Samaritan Drive, Corvallis, Oregon, using the HycelSuper 17 Hycell Incorporated, Houston, Texas

b. The first value for each parameter is pre-experimental

c. The second value for each parameter is post-experimental

Appendix Table 3. Recipes for the muffin and cocoa master mixes

Muffin Mix

Ingredient	Amount (kg)
Flour	34.02
Baking powder	1.47
Salt	0.64
Nonfat dry milk solids	5.07
Fat	10.98
Golden egg, powdered	2.70
Sugar	7.05

Cocoa Mix

Nonfat dry milk solids	13.62
Sugar	6.00
Cocoa	2.52
Salt	7.50 tsp

Appendix Table 4. Individual and group values for fecal vitamin B_6 (μmoles/3days)

	Group 1					
Date - Bran	(1)	(2)	(3)	(4)	(5)	Overall Mean ± SD
10/2	12.29	11.05	13.59	20.27	11.29	
10/5	12.88	14.07	8.57	16.37	14.60	
10/8	10.70	17.20	15.13	16.61	10.40	
10/11	12.17	12.29	8.51	10.99	9.57	
10/14	12.71	17.32	10.82	15.96	14.30	
10/17	12.53	11.82	8.33	11.88	9.40	
Mean ± SD	12.47 ± 0.27	13.81 ± 3.05	9.22 ± 1.39	12.94 ± 2.65	11.09 ± 2.78	11.91 ± 2.55
Non-bran						
10/20	10.93	12.23	9.46	15.60	8.10	
10/23	8.69	8.69	8.27	13.71	13.65	
10/26	9.22	7.68	6.97	9.69	7.62	
10/29	8.22	14.72	8.04	13.42	7.74	
11/1	9.40	11.29	8.04	12.12	7.62	
11/4	8.81	12.12	7.94	13.24	11.23	
Mean ± SD	8.81 ± 0.59	12.71 ± 1.79	8.01 ± 0.06	12.93 ± 0.70	8.86 ± 2.05	10.26 ± 2.44
Bran						
11/7	10.93	11.82	8.92	14.66	10.52	
11/10	10.22	13.24	9.16	11.35	11.70	
11/13	10.34	13.89	8.81	18.38	5.67	
11/16	9.57	11.23	7.83	13.48	15.83	
11/19	9.93	14.13	9.49	15.13	22.64	
11/22	7.44	7.62	8.39	10.05	4.42	
Mean ± SD	8.98 ± 1.35	10.99 ± 3.26	8.57 ± 0.84	12.89 ± 2.59	14.30 ± 9.21	11.15 ± 4.49

Table 4. (Continued)

Date - Non-bran	(6)	(7)	Group 2 (8)	(9)	(10)	Overall Mean ± SD
10/2	9.46	13.24	7.33	11.41	11.17	
10/5	6.03	11.41	9.69	7.27	11.17	
10/8	11.94	11.88	12.41	8.27	6.50	
10/11	7.92	12.65	14.60	8.51	7.33	
10/14	11.94	11.52	9.28	5.38	10.22	
10/17	8.81	9.10	17.55	7.33	7.92	
Mean ± SD	9.56 ± 2.11	11.09 ± 1.81	13.81 ± 4.19	7.07 ± 1.58	8.49 ± 1.53	10.00 ± 3.17
Bran						
10/20	10.64	10.46	18.56	7.56	10.28	
10/23	8.27	10.40	-	8.39	8.51	
10/26	10.58	13.53	14.18	8.57	12.06	
10/29	9.81	11.70	11.52	8.63	11.29	
11/1	10.34	9.10	14.89	8.63	9.34	
11/4	11.82	15.01	9.99	8.10	11.11	
Mean ± SD	10.66 ± 1.04	11.94 ± 2.96	12.13 ± 2.51	8.45 ± 0.31	10.58 ± 1.08	10.75 ± 2.08
Non-Bran						
11/7	10.70	11.58	12.00	7.51	7.45	
11/10	12.53	10.82	9.69	8.10	11.23	
11/13	9.69	10.34	12.00	7.09	12.12	
11/16	11.52	9.46	10.46	9.16	9.22	
11/19	11.23	10.04	14.54	7.45	10.70	
11/22	-	9.63	-	7.39	-	
Mean ± SD	11.37 ± 2.05	9.71 ± 2.98	12.50 ± 2.88	8.00 ± 1.01	9.96 ± 1.05	10.07 ± 1.91

Appendix Table 5. Individual and group values for urinary vitamin B_6 (μmoles/24 hours)

Date - Adjustment	Group 1 (1)	(2)	(3)	(4)	(5)	Overall mean ± SD
9/25	0.83	0.84	0.74	0.79	1.15	
9/29	0.89	0.84	0.84	0.72	1.35	
Bran						
10/2	0.98	0.81	0.87	0.82	0.97	
10/5	1.02	0.80	0.84	0.77	1.08	
10/8	0.99	0.93	0.88	0.85	0.83	
10/11	0.87	0.84	0.77	0.82	1.07	
10/14	0.86	0.77	0.68	0.76	0.97	
10/17	0.86	0.70	0.76	0.84	0.93	
Mean ± SD	0.85 ± 0.01	0.77 ± 0.07	0.74 ± 0.05	0.81 ± 0.04	0.99 ± 0.07	0.83 ± 0.10
Non-bran						
10/20	0.89	0.84	0.70	0.72	0.98	
10/23	0.89	0.80	0.69	0.82	0.92	
10/26	0.97	0.78	0.79	0.77	1.15	
10/29	1.02	0.80	0.72	0.83	1.19	
11/1	0.92	0.88	0.77	0.86	1.22	
11/4	1.00	0.81	0.75	0.83	0.89	
Mean ± SD	0.98 ± 0.05	0.83 ± 0.04	0.75 ± 0.03	0.84 ± 0.02	1.10 ± 0.18	0.90 ± 0.15
Bran						
11/7	0.87	0.74	0.72	0.81	1.07	
11/10	0.92	0.79	0.73	0.77	0.91	
11/13	0.96	0.81	0.64	0.74	0.93	
11/16	0.80	0.75	0.70	0.77	0.84	
11/19	0.87	0.72	0.66	0.81	0.92	
11/22	0.73	0.61	0.61	0.70	0.87	
Mean ±	0.80 ± 0.07	0.69 ± 0.07	0.66 ± 0.05	0.76 ± 0.66	0.88 ± 0.04	0.76 ± 0.09

Table 5. (Continued

Date - Adjustment	Group 2 (6)	(7)	(8)	(9)	(10)	Overall Mean ± SD
9/25	0.99	0.79	0.64	0.61	0.61	
9/29	0.89	0.92	0.53	0.63	0.63	
Non-Bran						
10/2	0.84	0.82	0.64	0.57	0.95	
10/5	0.83	1.00	0.85	0.58	0.96	
10/8	0.82	0.89	0.69	0.48	0.84	
10/11	0.74	0.78	0.70	0.56	1.11	
10/14	0.75	0.78	0.70	0.56	0.80	
10/17	0.70	0.70	0.80	0.55	0.80	
Mean ± SD	0.73 ± 0.03	0.75 ± 0.05	0.73 ± 0.06	0.56 ± 0.01	0.90 ± 0.18	0.73 ± 0.13
Bran						
10/10	0.67	0.75	0.63	0.53	0.84	
10/23	0.65	0.69	0.61	0.54	0.70	
10/26	0.68	0.79	0.73	0.53	0.99	
10/29	0.73	0.78	0.80	0.60	0.85	
11/1	0.69	0.73	0.67	0.59	0.91	
11/4	0.74	0.72	0.67	0.55	0.86	
Mean ± SD	0.72 ± 0.03	0.74 ± 0.03	0.71 ± 0.07	0.58 ± 0.03	0.87 ± 0.03	0.73 ± 0.10
Non-Bran						
11/7	0.66	0.70	0.63	0.56	0.85	
11/10	0.73	0.74	0.66	0.59	0.92	
11/13	0.75	0.73	0.67	0.60	0.80	
11/16	0.78	0.74	0.64	0.54	0.86	
11/19	0.76	0.68	0.65	0.57	0.84	
11/22	0.72	0.56	0.51	-	-	
Mean ± SD	0.75 ± 0.03	0.66 ± 0.09	0.60 ± 0.08	0.55 ± 0.02	0.85 ± 0.01	0.68 ± 0.11

Appendix Table 6. Individual and group values for urinary 4PA (μmoles/24 hours)

Date - Adjustment	Group 1 (1)	(2)	(3)	(4)	(5)	Overall Mean ± SD
9/25	5.87	3.69	4.48	3.16	5.29	
9/29	4.98	4.19	5.41	1.96	6.18	
Bran						
10/2	4.73	3.40	4.64	2.64	4.03	
10/5	5.08	5.05	4.40	2.25	4.25	
10/8	4.69	3.25	3.36	2.47	3.54	
10/11	4.89	3.05	3.77	2.49	4.83	
10/14	4.89	2.54	3.83	2.78	4.61	
10/17	4.44	2.64	4.02	2.37	4.05	
Mean ± SD	4.74 ± 0.26	2.74 ± 0.27	3.87 ± 0.13	2.55 ± 0.21	4.50 ± 0.40	3.68 ± 0.95
Non-bran						
10/20	5.50	3.17	4.05	2.45	4.26	
10/23	4.69	3.99	4.05	4.08	3.66	
10/26	4.85	2.97	4.91	2.65	3.92	
10/29	4.61	2.81	4.34	3.33	5.33	
11/1	3.23	3.76	4.48	3.84	5.35	
11/4	4.73	3.01	4.72	3.68	3.48	
Mean ± SD	4.19 ± 0.83	3.19 ± 0.50	4.51 ± 0.19	3.62 ± 0.26	4.72 ± 1.07	4.05 ± 0.81
Bran						
11/7	3.19	2.70	3.11	2.74	3.52	
11/10	3.97	2.48	4.17	2.72	3.03	
11/13	4.40	3.05	3.45	2.82	3.73	
11/16	4.09	2.70	3.86	2.90	4.05	
11/19	4.36	2.74	3.52	2.82	3.57	
11/22	-	2.72	3.38	3.05	3.32	
Mean ± SD	4.23 ± 0.19	2.72 ± 0.02	3.59 ± 0.25	2.92 ± 0.12	3.65 ± 0.37	3.36 ± 0.57

Table 6. (Continued)

Date - Adjustment	Group 2 (6)	(7)	(8)	(9)	(10)	Overall Mean ± SD
9/25	8.06	-	3.83	4.25	4.25	
9/29	6.99	4.63	3.34	5.03	5.03	
Non-bran						
10/2	6.18	5.03	3.09	4.42	1.33	
10/5	6.36	4.98	3.83	4.53	5.47	
10/8	5.36	4.72	3.40	3.22	5.01	
10/11	4.43	5.42	3.59	4.64	3.79	
10/14	5.15	4.81	3.67	4.51	3.73	
10/17	4.93	4.68	4.30	4.34	4.23	
Mean ± SD	4.84 ± 0.37	4.97 ± 0.39	3.85 ± 0.39	4.50 ± 0.15	3.92 ± 0.27	4.41 ± 0.55
Bran						
10/20	4.49	4.57	2.95	3.43	4.04	
10/23	4.34	4.28	3.03	3.25	2.67	
10/26	4.70	4.31	3.05	2.64	3.38	
10/29	4.52	4.68	3.79	4.20	3.29	
11/1	4.26	4.11	3.01	3.90	3.07	
11/4	4.52	4.63	3.66	3.69	3.71	
Mean ± SD	4.43 ± 0.15	4.47 ± 0.32	3.49 ± 0.42	3.93 ± 0.26	3.36 ± 0.33	3.94 ± 0.54
Non-bran						
11/7	4.93	4.42	3.25	4.29	-	
11/10	4.30	4.24	2.93	4.12	3.33	
11/13	4.39	4.50	3.57	4.16	2.91	
11/16	4.93	5.20	3.36	4.36	3.16	
11/19	4.51	4.15	3.71	3.93	3.29	
11/22	5.43	3.65	2.81	-	-	
Mean ± SD	4.96 ± 0.46	4.33 ± 0.79	3.29 ± 0.45	4.15 ± 0.30	3.23 ± 0.09	4.04 ± 0.82

Appendix Table 7. Individual and group values for plasma vitamin B_6 (nmoles/dl)

Date - Adjustment	(1)	(2)	(3) Group 1	(4)	(5)	Overall Mean ± SD
9/26	5.08	10.17	-	5.91	7.92	-
9/30	4.49	6.62	5.56	5.44	6.97	
Bran						
10/3	4.73	4.97	4.78	4.14	5.73	
10/6	4.25	4.61	4.14	3.90	5.08	
10/9	4.49	4.73	4.14	4.25	6.27	
10/12	5.08	5.79	4.37	4.85	5.79	
10/15	4.61	5.32	5.20	5.20	4.74	
10/18	4.73	4.85	3.90	4.25	4.61	
Mean ± SD	4.81 ± 0.24	5.32 ± 0.47	4.49 ± 0.66	4.77 ± 0.48	5.71 ± 1.07	5.02 ± 0.71
Non-bran						
10/21	4.85	5.67	4.37	6.15	4.97	
10/24	4.61	4.85	3.78	4.73	5.20	
10/27	5.91	5.79	4.73	5.91	6.03	
10/30	4.85	4.85	3.55	4.73	5.44	
11/2	5.20	5.91	3.78	5.32	5.44	
11/5	5.20	5.91	4.61	5.32	6.62	
Mean ± SD	5.08 ± 0.20	5.56 ± 0.61	3.98 ± 0.56	5.12 ± 0.34	5.83 ± 0.68	5.11 ± 0.78
Bran						
11/8	4.61	4.73	4.02	4.97	4.97	
11/11	3.78	4.85	3.31	4.73	4.25	
11/14	4.49	4.97	3.90	4.61	4.49	
11/17	3.90	4.85	4.25	4.85	4.25	
11/20	3.78	5.44	4.37	4.49	6.15	
11/23	3.90	5.44	4.49	4.96	5.79	
Mean ± SD	3.86 ± 0.07	5.24 ± 0.34	4.37 ± 0.12	4.77 ± 0.25	5.40 ± 1.01	4.73 ± 0.72

Table 7. (Continued)

	Group 2					
Date - Adjustment	(6)	(7)	(8)	(9)	(10)	Overall Mean ± SD
9/26	10.57	4.73	4.25	4.85	7.57	
9/30	6.15	4.73	2.48	3.31	7.92	
Non-bran						
10/3	4.97	4.91	2.60	3.43	6.62	
10/6	4.61	3.78	2.84	3.43	6.50	
10/9	4.73	4.49	3.19	3.43	5.79	
10/12	4.25	4.85	5.32	3.43	5.91	
10/15	5.67	5.20	3.90	3.78	5.79	
10/18	4.49	4.49	3.55	4.85	6.27	
Mean ± SD	4.80 ± 0.76	4.85 ± 0.35	4.26 ± 0.94	4.02 ± 0.74	5.99 ± 0.25	4.78 ± 0.90
Bran						
10/21	3.55	4.85	3.55	3.78	6.50	
10/24	2.84	4.02	2.60	3.90	6.86	
10/27	5.35	4.49	3.43	4.02	6.15	
10/30	3.19	3.78	3.43	3.43	5.44	
11/2	3.19	4.14	3.19	3.19	5.08	
11/5	3.66	4.25	3.31	3.07	5.08	
Mean ± SD	3.35 ± 0.27	4.06 ± 0.25	3.31 ± 0.12	3.23 ± 0.18	5.20 ± 0.21	3.83 ± 0.79
Non-bran						
11/8	3.90	4.49	3.55	3.61	4.85	
11/11	3.55	3.90	3.55	3.66	4.61	
11/14	3.43	4.37	2.95	3.55	5.08	
11/17	4.61	4.49	3.96	4.14	4.85	
11/20	3.90	4.25	3.55	3.78	5.32	
11/23	4.37	4.25	4.02	3.66	4.96	
Mean ± SD	4.29 ± 0.36	4.33 ± 0.14	3.84 ± 0.25	3.86 ± 0.25	5.04 ± 0.25	4.27 ± 0.50

Appendix Table 8. Individual and group values for plasma PLP (nmoles/dl)

	Group 1					
Date - Adjustment	(1)	(2)	(3)	(4)	(5)	Overall Mean ± SD
9/26	3.68	6.36	-	4.10	7.60	
9/30	3.65	5.61	5.22	4.31	5.58	
Bran						
10/3	3.04	3.68	4.41	3.18	4.66	
10/6	3.63	3.71	3.92	2.91	4.93	
10/9	2.70	3.31	3.69	2.86	5.83	
10/12	3.60	3.92	2.90	3.26	5.99	
10/15	2.80	3.83	3.67	3.10	3.72	
10/18	3.31	4.82	3.45	3.32	3.52	
Mean ± SD	3.24 ± 0.41	4.19 ± 0.55	3.34 ± 0.40	3.23 ± 0.11	4.41 ± 1.37	3.68 ± 0.80
Non-bran						
10/21	3.15	4.83	3.53	4.15	4.10	
10/24	3.79	4.73	3.85	3.81	4.00	
10/27	4.19	4.65	3.73	4.21	4.79	
10/30	4.05	3.76	3.60	4.71	4.33	
11/2	3.77	4.80	3.72	4.64	5.32	
11/5	3.39	4.25	4.24	4.67	5.70	
Mean ± SD	3.74 ± 0.33	4.27 ± 0.52	3.85 ± 0.34	4.67 ± 0.03	5.12 ± 0.71	4.33 ± 0.65
Bran						
11/8	3.09	3.78	3.41	4.03	3.58	
11/11	2.86	4.23	4.07	4.17	4.00	
11/14	3.43	4.58	4.07	3.77	4.51	
11/17	2.59	4.47	4.34	3.89	4.24	
11/20	3.08	5.21	4.03	3.75	5.89	
11/23	3.05	4.22	3.54	3.66	3.55	
Mean ± SD	2.91 ± 0.27	4.63 ± 0.51	3.97 ± 0.40	3.77 ± 0.12	4.56 ± 1.20	3.97 ± 0.84

Table 8. (Continued)

Date - Adjustment	Group 2 (6)	(7)	(8)	(9)	(10)	Overall Mean ± SD
9/26	9.26	4.95	3.46	4.11	7.24	
9/30	4.84	4.89	2.48	3.22	6.70	
Non-bran						
10/3	4.04	4.27	2.31	2.54	4.83	
10/6	3.81	3.66	2.48	2.80	3.40	
10/9	2.99	3.37	2.78	2.19	3.79	
10/12	2.71	3.79	2.46	2.49	4.21	
10/15	2.88	3.36	2.60	2.35	4.90	
10/18	3.09	3.67	2.40	2.41	4.61	
Mean ± SD	2.89 ± 0.19	3.61 ± 0.22	2.49 ± 0.10	2.42 ± 0.07	4.57 ± 0.35	3.19 ± 0.85
Bran						
10/21	2.33	4.11	2.14	2.34	4.68	
10/24	1.99	3.61	1.99	2.75	4.79	
10/27	2.57	3.62	2.46	3.03	4.58	
10/30	2.63	2.92	2.42	2.84	4.59	
11/2	2.63	3.13	2.58	2.82	3.85	
11/5	2.29	2.91	2.67	2.49	4.13	
Mean ± SD	2.52 ± 0.20	2.99 ± 0.12	2.56 ± 0.13	2.72 ± 0.20	4.19 ± 0.37	2.99 ± 0.67
Non-bran						
11/8	2.50	3.01	2.33	3.18	3.86	
11/11	2.76	3.29	2.78	3.17	3.49	
11/14	2.82	3.64	2.50	2.46	3.30	
11/17	2.68	3.47	2.46	2.63	3.49	
11/20	2.33	3.27	2.70	2.98	4.00	
11/23	2.76	3.96	2.98	2.70	3.81	
Mean ± SD	2.59 ± 0.23	3.57 ± 0.35	2.71 ± 0.26	2.77 ± 0.19	3.77 ± 0.26	3.08 ± 0.55

Appendix Table 9. Individual and group values for creatinine (g/24 hours)

Date - Adjustment	(1)	(2)	(3)	(4)	(5)	Overall Mean ± SD
9/25	1.59	1.46	1.11	1.51	2.14	
9/29	1.68	1.53	1.45	1.25	2.79	
Bran						
10/2	1.60	1.45	1.47	1.55	2.05	
10/5	1.63	1.53	1.50	1.46	2.13	
10/8	1.68	1.63	1.50	1.61	1.96	
10/11	1.68	1.65	1.45	1.61	2.18	
10/14	1.74	1.66	1.61	1.55	2.10	
10/17	1.68	1.59	1.51	1.85	2.16	
Mean ± SD	1.70 ± 0.03	1.63 ± 0.04	1.52 ± 0.08	1.67 ± 0.16	2.15 ± 0.04	1.73 ± 0.23
Non-bran						
10/20	1.64	1.73	1.54	1.59	2.13	
10/23	1.64	1.71	1.58	1.77	2.15	
10/26	1.64	1.50	1.58	1.61	2.05	
10/29	1.14	1.64	1.50	1.63	2.11	
11/1	1.62	1.73	1.62	1.66	2.52	
11/4	1.78	1.62	1.54	1.64	1.93	
Mean ± SD	1.51 ± 0.33	1.66 ± 0.06	1.55 ± 0.06	1.64 ± 0.01	2.19 ± 0.03	1.71 ± 0.31
Bran						
11/7	1.66	1.56	1.59	1.53	2.13	
11/10	1.72	1.57	1.61	1.57	1.93	
11/13	1.66	1.65	1.55	1.65	2.08	
11/16	1.66	1.63	1.64	1.64	2.05	
11/19	1.60	1.58	1.47	1.57	1.77	
11/22	1.60	1.74	1.66	1.61	2.21	
Mean ± SD	1.62 ± 0.03	1.65 ± 0.08	1.59 ± 0.10	1.61 ± 0.03	2.01 ± 0.22	1.69 ± 0.19

Table 9. (Continued)

Date - Adjustment	(6)	(7)	(8)	(9)	(10)	Overall Mean ± SD
9/25	2.10	1.87	1.42	1.23	1.69	
9/29	2.18	1.98	1.24	1.27	2.72	
Non-bran						
10/2	1.97	2.14	1.39	1.27	2.61	
10/5	2.05	2.01	1.50	1.05	1.45	
10/8	2.03	2.03	1.57	1.31	2.14	
10/11	2.10	1.72	1.63	1.28	1.61	
10/14	2.11	2.00	1.49	1.30	1.77	
10/17	2.04	1.91	1.88	1.37	1.75	
Mean ± SD	2.08 ±0.04	1.88 ±0.14	1.67 ±0.20	1.32 ±0.05	1.71 ±0.09	1.73 ±0.28
Bran						
10/20	2.20	2.00	1.57	1.26	1.74	
10/23	2.04	1.84	1.49	1.27	1.01	
10/26	2.05	1.97	1.47	1.23	1.89	
10/29	2.20	2.01	1.65	1.35	1.75	
11/1	1.96	1.87	1.57	1.41	1.75	
11/4	2.14	2.01	1.71	1.36	2.11	
Mean ± SD	2.10 ±0.12	1.96 ±0.08	1.64 ±0.07	1.37 ±0.03	1.87 ±0.21	1.79 ±0.28
Non-Bran						
11/7	1.97	1.91	1.63	1.23	1.67	
11/10	2.13	1.96	1.61	1.32	1.68	
11/13	1.87	1.89	1.48	1.34	1.77	
11/16	2.17	2.09	1.56	1.09	1.70	
11/19	2.04	2.04	1.54	1.22	1.71	
11/22	2.16	1.98	1.49	-	-	
Mean ± SD	2.12 ±0.07	2.04 ±0.05	1.53 ±0.04	1.24 ±0.10	1.71 ±0.01	1.75 ± 0.35