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LONGITUDINAL PANTOTHENIC ACID STATUS OF

PREGNANT AND LACTATING WOMEN

by

Won Oack Song

A dissertation submitted in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

Approved:

UTAH STATE UNIVERSITY P Logan, Utah

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Won O. Song

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ABSTRACT

Longitudinal Pantothenic Acid Status of Pregnant and Lactating Women

by

Won Oack Song, Doctor of Philosophy Utah State University, 1983

Major professors: Dr. Bonita W. Wyse and Dr. R. Gaurth Hansen Department: Nutrition and Food Sciences

Pantothenic acid nutritional status was evaluated in a cohort of twenty-nine Caucasian pregnant women, aged 20-35, during their third trimester of pregnancy, at two-weeks postpartum and at three months postpartum. Seventeen non-pregnant, non-lactating women who had similar demographic backgrounds as the pregnant women were selected as a control group and followed the same experimental schedule as the pregnant women. A fasting blood sample, two days 24-h urine specimens and diet record were obtained for each of two days from each subject at each period.

The mean dietary pantothenic acid intakes of the women during pregnancy $(5.30^{+}1.74 \text{ mg/day})$ and during lactation $(8.90^{+}11.66 \text{ mg/day})$ were statistically similar to that of the control group

 $(9.63^{+}19.74 \text{ mg/day})$. A substantial number of the study population consumed less than 4 mg pantothenic acid daily. The average of 2.75 mg dietary pantothenic acid intake per 1000 kcal was consistent in all groups. The mean fasting blood level (406.38⁺78.21 ng/ml) and urinary excretion $(3.21 \pm 0.88 \text{ mg/day}, 3.85 \pm 1.24 \text{ mg/g creatinine})$ of the pregnant women were significantly lower than those of the control group $(530.14^{+}157.90 \text{ ng/m}); 4.38^{+}3.69 \text{ mg/day}, 5.78^{+}4.90 \text{ mg/g}$ creatinine). The suppressed panthothenic acid levels in blood and urinary excretion during pregnancy were increased to levels comparable to controls during the nursing period. Pantothenic acid levels in the biological fluids correlated significantly with dietary intake. A bound form of pantothenic acid was not detected in the plasma and plasma values were relatively constant. Thus, plasma was suggested as an insensitive indicator of pantothenic acid nutritional status. The mean pantothenic acid contents of fore and hind milk samples were 2.60 microgram/ml, 2.44 microgram/ml, respectively and were not statistically different. Overall, the pregnant women consumed less than the suggested level of pantothenic acid, maintained lower blood levels and had decreased urinary excretion.

(157 pages)

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CHAPTER I

INTRODUCTION

Maternal nutrition during pregnancy and lactation plays a significant role for both the mother and her infant. A good diet is necessary for maternal tissue maintenance, for growth and development of the fetus, for replenishment of maternal nutrient stores and for producing breast milk of proper quantity and quality. Studies (NAS, 1970; Pernoll, 1974; Worthington-Roberts et al., 1981; Metcoff et al., 1981; Falkner, 1981; Raman, 1981; Susser, 1981; Pratt et al., 1951) have shown that changes in maternal diet influence fetal growth and the nutrient level of breast milk, especially certain water soluble vitamins.

Breast feeding is believed to benefit the child as well as the mother for several reasons including nutritional, immunological, psychological, physiological and economical advantages. With increasing understanding of the importance of human milk as a food for infants, the number of women initiating breast feeding has increased to 54% of all mothers giving birth in the United States in 1980 (Martinez et al., 1981).

Nutrient allowances for the maternal population are 25% to 100% higher for all nutrients than those for non-pregnant and non-lactating women (RDA, 1980). A Recommended Dietary Allowance (RDA) for pantothenic acid has not yet been established. Instead, in the 1980 revision of the RDA the Food and Nutrition Board of the National Academy of Sciences and National Research Council (RDA, 1980) established the "Estimated Safe and Adequate Daily Dietary Intake" of 4-7 mg pantothenic acid for adults and an undefined higher intake for pregnancy and lactation. The provisional estimations are based on a limited number of pantothenic acid status studies. Estimated adequate intake of pantothenic acid for other age groups, including infants, are based on proportional energy needs.

Pantothenic acid is a structural component of coenzyme A (CoA) and acyl carrier protein (ACP) which play vital roles in more than 70 different steps of carbohydrate, fat and amino acid metabolism and in the synthesis of many vital compounds such as steroids, porphyrine, and acetyl choline (Baker and Frank, 1968; Abiko, 1975). Pantothenic acid essentiality is well established in human beings and animals for sustaining life, reproduction capacity and normal growth (Hodges, et al., 1959; Nelson and Evans, 1946; Hurley et al., 1965). A deficiency depresses humoral antibody responsiveness to various antigens in experimental animals and in men (Beisel, 1982).

Studies on pantothenic acid status in pregnant and lactating women reported that pregnant teenagers were consuming only an average of 4.7 mg/day (Cohenour and Calloway, 1972). Indian pregnant and nursing women had lower levels of the vitamin in blood and urinary excretion than a control population did (Srinivasan and Belavady, 1976) and Japanese pregnant women had lower blood levels than did a comparative group of non-preganant women (Ishiguro, 1962). Many studies have also indicated a positive correlation between the vitamin level in breast milk and maternal dietary intake (Johnston et al., 1981; Deodhar and Ramakrishnan, 1960). The data suggested that this nutritionally high risk group was generally in unsatisfactory status.

Although the number of the breast-fed babies is increasing in the United States, only a few study have reported the pantothenate content of individual or pooled human milk samples. Some of the studies were done abroad and/or in lower socioeconomic groups; the others were reported about 30 years ago in this country.

There is a dearth of literature on the pantothenic acid status in gravid and nursing women and the vitamin content of their breast milk. Thus, together with earlier related works, knowledge of an average pantothenate dietary intake, urinary excretion, blood level in gravid and nursing women and concomitant determination of the vitamin content in their milk could be used as a guide in determining recommended allowance for the population and the composition of infant formulas.

Objectives

 To assess pantothenic acid status of pregnant and nursing women longitudinally with dietary and biochemical parameters.

2. To determine pantothenate content in mature human milk.

3. To examine the relationship between maternal nutritional status and milk content.

4. To evaluate changes in dietary intake of twenty six other

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nutrients along with physiological changes.

CHAPTER II

REVIEW OF LITERATURE

General Maternal Status

For a full term pregnancy, approximately 80,000 kcal are required for growth of the fetus and accessory tissues, and increased maternal metabolism (Hytten and Leitch, 1971). This gross energy amounts to an additional of 300 kcal to the daily allowance for non-pregnant women. During the first three months of lactation, an average daily production of 850 ml of breast milk increases the maternal energy requirement by about 1000 kcal per day. Of the energy increment, about 500 kcal are taken as extra energy from the diet and the rest is drawn from the 2-4 kg of extra maternal adipose tissue stored during normal pregnancy. During this period CoA plays an important role in oxidizing adipose fats for the extra calorie needs.

The Food and Nutrition Board of the National Academy of Science and National Research Council recommends (RDA, 1980) an increased intake of all vitamins and minerals during pregnancy and lactation from 25% more for vitamin B6 and folacin to as much as 100% more for vitamin D and 300% more for iron than for non-nursing and non-pregnant women. The increased allowances for vitamin C, thiamin, riboflavin, niacin and vitamin B_{12} are 80%, 50%, 41%, 38%, and 33% of the daily

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allowance for the non-pregnant women respectively (RDA, 1980).

Information on general nutritional status of gravid and lactating women is not abundant. Roepke and Kirksey (1979) reported on vitamin ${\rm B}_6$ nutriture during pregnancy and lactation. They found that dietary intakes of vitamin B6 were significantly correlated with levels of the vitamin in maternal serum, in cord serum and in milk. The mean dietary intake of vitamin B_6 for all subjects studied was 1.24 mg/day, which was approximately 50% of the RDA for pregnancy. Mean intakes of iron, magnesium and zinc from the diet only were less than two-thirds of the RDA. Sims (1978) examined dietary practice of 61 nursing mothers, a white, middle class and highly educated group, who had been breast-feeding their infants for an average of 4 months, and found that 80% of the group were taking vitamin supplements. Among the women taking no supplement, 91% of the participants failed to meet the RDA for energy, 64% failed to meet the RDA for calcium, vitamin A, riboflavin and niacin, 45% failed to meet the RDA for thiamin and 100% failed to meet the RDA for iron. Similar results were observed in the pregnant population by Lillien et al. (1982) who reported that only 30 % of the population studied had intakes of at least two-thirds of RDAs for the group. Even though the studies did not include pantothenic acid, lower intake of the vitamin can be suspected among women taking no supplements.

Sneed et al. (1981) examined four water soluble vitamins in a group of lower socioeconomic lactating women in U.S. and found that the dietary intake of the group was low in vitamin B_6 and folate.

Their milk composition was significantly changed after the maternal diet was supplemented with vitamin B_6 , vitamin B_{12} and folate. Because of consistently low levels of the water soluble vitamin in the milk of the group, the authors suggested that either dietary change or supplements were necessary to maintain recommended levels of the vitamins in the women's breast milk.

The extent and timing of the increase in maternal nutrient requirement vary considerably from nutrient to nutrient. Roepke and Kirksey (1979) indicated that 5 months gestation was a critical time for the assessment of maternal vitamin B_6 nutriture; Metz (1970) reported folate deficient megaloblastic anemia occurred in nursing subjects because the supply of the nutrient to the breast milk takes precedence over maternal needs even when the mother was severely folate deficient.

Studies indicated lower blood or serum levels of many water-soluble vitamins in long-term oral contraceptive users. Previous usage also seemed to affect adversely nutritional status during pregnancy (Roepke and Kirksey, 1979). Lewis and King (1980), however, found that the effect of oral contraceptive use on the change in biochemical parameters can be real or an artifact dependant on the time of sampling in the menstrual cycle. The authors observed that both urinary and blood pantothenate levels of oral contraceptive users were significantly depressed during the first week of their menstrual cycle when compared to those of non-users. However, the difference was insignificant when samples were collected at the 12th day. Recommendations of vitamin and mineral supplements for pregancy and lactation are conflicting among nutrition textbooks (Guthrie, 1979; Gibbs and Seitchik, 1980; Worthington-Roberts et al., 1981). Guthrie (1979, p.432) states

In light of the very high level of nutritional intake prescribed for normal lactation, it is likely sound to recommended for the mother the use of a dietary supplement that provides protective levels of nutrients for which the possibility of a dietary lack exists.

Gibbs and Seitchik (1980, p.751) indicate

Most of the additional requirements for lactation can be met by a quart of milk a day.

Worthington-Roberts et al. (1981, p.150) reports that

Although lactation increases a woman's requirement for nearly all nutrients, these increased needs can be provided by a well balanced diet. For this reason nutritional supplements are generally unnecessary except when there is a deficient intake of one or more nutrients.

Concerns about Pantothenic Acid

Status Study

Research on pantothenic acid nutriture in human beings has been limited for several reasons. Since pantothenic acid is ubiquitous in nature a deficiency has been rare. An overt dietary deficiency has not been clinically recognized in man except when a metabolic antagonist, omega-methyl pantothenic acid, was administered (Hodges et al., 1959) or when a pantothenic acid free semi-synthetic diet was given for 10 weeks (Fry et al., 1976). The deficiency symptoms observed in human beings were: 1) a neuromotor disorder; 2) cardiovascular instability, especially in the upright position; 3) complaints referable to the gastrointestinal system; 4) repeated infections; physical and mental depression and 6) alteration in biochemical functions including acetylation, carbohydrate metabolism, blood cholesterol, steroid hormon secretion, plasma protein and failure of ACTH to induce eosinopenia (Bean et al., 1955). Deficiency symptoms observed in animals (Novelli, 1953; Hurley et al., 1965) included infertility, spontaneous abortion, frequent neonatal death, retarded growth rates in young animals, sudden death, abnormalities in skin, hair and feathers, neuromuscular disorders, gastrointestinal malfunction and adrenal cortical failure.

Concern about pantothenic acid status for today's population partly stems from increased intake of processed and refined foods. The nutrient loss resulting from processing ranges from 7% in canned fruit juice to 78% in canned vegetables (Schroeder, 1971). The loss of pantothenic acid in dairy products averages 30-35% (Goerner and Uherova, 1980; Schroeder, 1971). Pantothenic acid content of breads and cereals is reduced to 50% of the original content when it is milled to 70% extraction flour. The vitamin is stable in neutral solution but not in heat, alkali or acid.

Pantothenic acid deficiency has been recognized during long term use of total parenteral nutrition (TPN) (Chipponi et al., 1982). Leevy et al. (1965) also observed over twenty-five percent of the municipal hospital population had a reduction in circulating pantothenate blood level. Interestingly, over two-thirds of those with low circulating levels of pantothenic acid had a normal dietary history. The authors classfied a value of 20% less than the lower range of values, less than 5 ng panthothnate per ml of blood, as abnormal.

The extent to which suspected synthesis of pantothenate by intestinal microflora contributes to body needs appears to be negligible. The results of three balance studies (Oldham et al., 1946; Gardner et al., 1943; Denko et al., 1946) indicated that urinary plus fecal excretion almost equaled (93%, 102%, 112% respectively) total dietary intake. Although these data may need careful interpretation because none of the studies used the duo-enzyme treatment to release bound pantothenate from samples as is now done, the possibility of enteric synthesis seems slight.

Technical difficulties have existed with the traditional methodology for measuring the vitamin in a wide range of biological samples and foods. Research on pantothenic acid nutritional status and metabolism had been hindered by the tedious microbiological assay until a radioimmunoassay became available (Wyse et al., 1979). Although spectrophotometric methods had existed previously their usage has been limited to pharmacological preparations.

Comparisons of the published data from different laboratories have been inconsistent and the discrepancies have seldom been accompanied by explanations. Several of the followings have been

speculated as the reason for the large disagreement and variation among the published data. First, a large subject variation in blood, urine, plasma, milk and diet intake has been reported within studies. Second, various procedures for enzyme hydrolysis have been used by different researchers. Since the available assays are sensitive only to free pantothenic acid, prior enzymic liberation of pantothenic acid from bound forms are essential. For this purpose, more than a dozen enzymes have been used by different investigators since the discovery of the CoA. A simultaneous double enzyme treatment with avian liver extract and alkaline phosphatase has been most commonly employed. Baker et al. (1975, 1969), however, have consistently used clarase for the purpose, claiming that the single enzyme produced preferred results over the double enzyme treatment. They have reported lower pantothenic acid values than other groups. Third, the quality and quantity of enzymes employed can contribute to the variation in data. The traditionally used avian liver extract has been found to contain large quantities of endogenous pantothenic acid and are not specific for the amide bond between the pantothenate and cysteamine (Wittwer, 1982). Fourth, different procedures for deproteinization can also increase the variation because many of these produce opaque aliquots which can interfere with the assays. Fifth, the assumption that the traditional double enzymes liberate all of the bound form of pantothenate in biological tissues or that all pantothenate is present as CoA or its breakdown products in tissue has been erroneous. Marjerus et al. (1965) found that the dual enzyme did not hydrolyze

the phosphodiester bond between the phosphopantethein and serine molecule in ACP. Last, other bound forms of pantothenic acid such as glycosyl derivatives found in tomato and its products (Amachi et al., 1970) are unlikely catalyzed by the enzymes.

The traditional methodology also slowed the completion of food composition tables (Zook et al., 1956; Orr, 1969) on which most of the pantothenic acid status studies are based for calculation of dietary intake.

Means for detecting and evaluating pantothenic acid deficiency are limited. Even though CoA, a metabolically active form of pantothenic acid, functions as an acyl group activator in over 70 different metabolic reactions and is present in all human tissues (Lehninger, 1975; Abiko, 1975), it has not been easy to isolate a metabolic step that can sensitively reflect nutritional status of the nutrient. Findings from dietary histories thus often do not correlate well with clinical signs or biochemical changes of nutritional status.

Recently pantothenic acid and its derivatives have been implicated in altered metabolism. We know very little about the dynamic process of the biosynthesis and degradation of CoA and ACP. The enzymic control point of the mechanism remains unknown.

Panthothenic acid status in many altered metabolic states are of interest. Circulating levels of the vitamin are depressed in alcoholics (Leevy et al, 1965; Tao and Fox, 1976^b; Markkanen, 1973), in arthritics (Barton-Wright and Elliott, 1963), in starvation (Leevy et al., 1965), in the aged (Ishiguro, 1972) and in the hypertensive (Saito and Hareyama, 1970; Koyanagi et al., 1967); no difference is observed in patients with ulcerative and granulomatous colitis (Ellestad-Sayed et al., 1976) and in patients with metabolic disease, diseases of the thyroid gland and diabetes (Markkanen, 1973); a higher level has been reported in serum of diabetic rats (Reibel et al., 1981).

Several pharmacological effects of pantothenic acid and its derivatives in experimental animals and human beings have been reported. Shirai et al. (1979) and Shinomiya et al. (1980) observed in rats a decrease in cholesterol ester synthesis after administration of pantetheine. Similar biochemical effects were observed after Clofibrate was administered to experimental rats (Hilkka et al., 1979; Skrede and Halvorsen, 1979). The use of pantetheine for prevention of atherosclerosis was suggested by Nakamura (1977) and Hidemasa (1977).

<u>Previous Studies with Pregnant</u> and/or Lactating Women

Cohenour and Calloway (1972) studied the diets of seventeen pregnant teenagers and found that an average daily intake was 4.7 mg. Only seven of the seventeen pregnant girls consumed more than 5 mg (ranged 1.8-7.2 mg) without vitamin supplements. Postpartum girls, none of whom was lactating, consumed an average of 4.1 mg pantothenic acid per day (ranged 1.9-6.5 mg). The dietary intake was obtained by calculation based on the work of Zook et al. (1956) supplemented by

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those of Orr (1969). The researchers examined the level of the vitamin in urine and in whole blood and found that the pregnant group had lower pantothenate levels in blood than non-pregnant girls had. The blood levels of pantothenic acid in pregnant, postpartum and non-pregnant girls were 1030 ng/ml, 1120 ng/ml and 1830 ng/ml respectively. The authors concluded that the study group was in unsatisfactory pantothenic acid status.

Ishiguro (1962) found an average of 655 ng/ml of pantothenic acid in whole blood of third trimester Japanese pregnant women, aged 20-30; whereas a group of non-pregnant women had an average level of 966 ng/ml. This study did not include information on dietary intake of the vitamin, thus the data cannot be compared with those of Cohenour and Calloway (1972).

In a study of ten lactating women (Pratt et al., 1951), in which participants were not recieving supplements, an average daily intake of 8.03 mg pantothenic acid and 24 h urinary excretion of 4.47 mg (ranged 2.20-6.17 mg) were reported. Johnston et al. (1981) reported that the mean pantothenic acid intake from the diet of 22 lactating women was 5.8 mg/day over a 6 month period. The intake from diet plus supplement was 6.7 mg which is in the range of estimated allowance for normal adults.

Srinivasan and Belavady (1976) investigated the vitamin levels in blood of pregnant and nursing Indian women of a lower socioeconomic status and found average levels of 570 ng/ml and 634 ng/ml in whole blood and 169 ng/ml and 193 ng/ml in plasma in respective group. These concentrations were comparable with 626 ng/ml in whole blood and 197 ng/ml in plasma of a control group. The mean urinary excretion of 24 h collection in the gravida and the control were 2.0 mg and 2.5 mg respectively and the difference was larger when expressed in mg/g creatinine (2.8 mg/g and 3.8 mg/g creatinine respectively). The authors found that at parturition infant cord blood contained significantly higher concentration, 943.8 ng/ml, than did the maternal blood, 662.1 ng/ml. The same relation was found by Baker et al. (1975) and Cohenour and Calloway (1972). Baker et al. (1975) reported a mean of 995 ng/ml (ranged 451-1700 ng/ml) in new born blood and an average of 250 ng/ml (ranged 200-800 ng/ml) in maternal blood. Cohenour and Calloway (1972) reported 1440 ng/ml in the newborn blood and 1030 ng/ml in the maternal blood.

The published data on pantothenic acid level in blood, urine, milk and dietary intake in women are summarized in Table 1.

Previous Studies on Human Milk

Water Soluble Vitamins

The composition of milk changes with eatablishment and progression of lactation. The concentration of some constituents increase and of others decrese as mammary structures adjust to the demands of milk production. The milk secreted by different

Poforonco	Subjects	Panto			
Ke l'el elice	50050000	Intake mg/day)	Blood (ng/ml)	Urine (mg/day)	Milk
Pratt et al. (1951)	10 lactating women	8.03	ND	4.47	1.72 mg/day
Fox & Linkswiler (1961)	8 women, ages18-24	6.7	ND	3.9	ND
Ishiguro (1962)	pregnant women (Japanese)	ND	725	ND	ND
Ishiguro (1972)	women,ages 40-44 (Japanese)	ND	970	ND	ND
Cohenour & Calloway (1972)	17 pregnant teenagers supplements	4.7 ⁺ 1.4 (1.8-7.2)	1030 (600-1450)	ND	ND
	<pre>13 pregnant teenagers, 14 postpartum teenagers nonlactating 5 non-pregnan women</pre>	7.2 ⁺ 1.9 (4.6-10.4) 4.1 ⁺ 1.1 (1.9-6.5)	1120 (850-1720)	3.5 (1.0-5.2)	ND
		t 3.3 ⁺ 2.2 (1.1-6.9)	1830 (1050-2420	2.5) (1.4-3.5)	ND
Baker et al.	76 non-pregna	nt ND	500	ND	ND
(19/3)	Nomen 174 women at parturition	ND	450	ND	ND

Table 1. Pantothenic acid dietary intake of women and its levels in blood, urine and human milk reported in the literature.

Table 1. Continued

Contractory of the second s		Pa	ntothenic Aci	d Level	
Reference	Subjects				
		Intake	Blood	Urine	Milk
		(mg/day)	(ng/ml)	(mg/day)	
Srinivasan & Belavady	57 pregnant women	ND	570	2.0	ND
(1976)	24 lactating women(1-6 mo	ND)	634	ND	2.3
microgram/n	nl				
	14 non- pregnant wom (Indian, low socioeconom	ND en er ic group)	626	2.5	ND
Johnston	22 lactating	women:			
et al. (1981)	Dietary Diet +	5.8	ND	ND	ND
	supplement Average	22.3 7.6	ND ND	ND ND	ND 6.7
microgram/m	1]				

Numbers in parenthesis are ranges. ND: no data Mean-standard deviation

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individuals varies in composition between wide limits. Both the quality and quantity of milk may be in some degree altered by hereditary factors, amount and kinds of food consumed, environmental factors (work, rest, etc.), disease and emotional status of the subject.

Many studies have reported that content of water soluble vitamins in human milk is influenced by maternal dietary intake (Thomas et al., 1979; West and Kirksey, 1976; Tamura et al., 1980; Nail et al., 1980; Cooperman et al., 1982; Sneed et al., 1981). In a folate study, Cooperman et al. (1982) reported that supplementation with folic acid to folate deficient women resulted in a prompt increase in milk folate level. The authors observed the folate level was low in colostrum but the concentration increased as lactation proceeded. The vitamin presented in milk was in a well-absorbable form because hydrolysis with an enzyme, conjugase, did not release additional folate. West and Kirksey (1976) reported that subjects consuming less than the RDA of vitamin B₆ produced milk with significantly lower content of the vitamin. Marked diurnal variation in the vitamin B₆ content of milk was observed among individuals taking daily supplements of the vitamin with peak levels occuring 3-5 h after the supplements were taken. Thomas et al. (1979) reported that even when maternal blood levels of vitamin B_6 and vitamin B_{12} were in the normal range, supplementation with the vitamins significantly increased concentration of the vitamin in milk. Nail et al. (1980) reported that riboflavin level in milk was reflected by supplementation whereas thiamin was not.

Concentration of the both vitamins was significantly increased as the lactation progressed through the first six weeks postpartum. Sneed et al. (1981) reported that supplementation of vitamin B_6 , vitamin B_{12} and folate to the maternal diet significantly increased the vitamin concentration of milk. The authors believed that either dietary changes or supplements may be necessary to maintain recommended levels of the milk vitamins in the lower socioeconomic lactating women.

Pantothenic Acid.

Pantothenic acid allowance has not been established for infants based on human milk composition and balanced studies as for other water soluble vitamins. The provisional estimated adequate intake for the vitamin for infants is rather based on proportional energy needs (RDA, 1980).

The average pantothenic acid content in mature human milk varies from 1.6 microgram/ml in data of Williams et al. (1942) to 6.9 microgram/ml in data of Johnston et al. (1981) as summarized in Table 2. The individual variation is even larger and ranges from 0.8 microgram/ml to 7.6 microgram/ml among the published data. Data of Johnston et al. (1981) in particular disagree with other studies. The authors speculated that their large values might be due to their enzyme treatment in which a double enzymes of intestinal phosphatase and pigeon live extract was used to release free pantothenic acid from the bound form. Yet the data still disagree with those of the Department of Health and Social Security, England (1977) and Deodhar and Ramakrishnan (1960) who used the same treatment for their samples.

In the study of Pratt et al. (1951), lactating women were given multivitamins containing approximately ten times the RDA (1948) of vitamin A, vitamin C, and all B vitamins except pantothenic acid. They found no changes in the secretion of pantothenic acid in breast milk nor the excretion in urine during the period of several days and suggested pantothenic acid probably responded independently from the other vitamins in occurance in milk. Pantothenic acid content, however, changed with the progression of lactation. Coryell et al. (1945) found the pantothenic acid level in colostrum (1-5 days postpartum) of 0.48 microgram/ml while those of transitional (6-10 days postpartum) and mature milk (after 10 days postpartum) were 2.45 microgram/ml and 3.04 microgram/ml respectively. Unlike thiamin and niacin, pantothenic acid content rose abruptly during the first three or four days postpartum. A similar trend was observed by Srinivasan and Belavady (1976). In the latter study, pantothenic acid content in colostrum (1-10 days postpartum) of a lower socioeconomic group of Indian lactation women was 1.3 microgram/ml versus those of later stages were 2.2 to 2.3 microgram/ml. Pellegrini and Chiari (1955) also reported lower values in colostrum (1.10 - 1.42 microgram/ml) than in mature milk with an average of 2.54 microgram/ml. In these studies, levels decreased or leveled off somewhere between the 10th day and second month postpartum.

A correlation between maternal dietary intake and pantothenic acid content of human milk was examined by a few investigators.

Johnston et al. (1981) reported an average pantothenic acid concentration of 6.7 microgram/ml in human milk and a correlation (r=0.51) between the pantothenic acid intake and its content of milk. No significant difference was observed between the pantothenic acid levels of fore and hind milk and during the progression of nursing. A similar correlation (r=0.40) was found by Deodhar and Ramakrishnan (1960) between the pantothenic acid intake of 60 women and the vitamin content of their milk. Daily pantothenic acid intakes grouped into means of 1.83 mg, 3.33 mg, 5.02 mg and 8.65 mg yielded vitamin levels in milk of 1.02, 1.36, 1.65 and 1.84 microgram/ml respectively. The authors further persued the effect of the vitamin supplements on the milk level (Deodhar et al., 1964). In the study the daily dietary intake was increased from 2.2 mg of pantothenic acid from food only with an additional 50 mg supplementation during an eight month period with a gradual increase each month. The pantothenic acid content of milk increased accordingly, starting from 1.0 microgram/ml of milk with no supplementation to 3.03 microgram/ml with 50 mg dietary supplementation. Schmidt (1950) reported a diurnal variation in the vitamin level of milk following the oral administration of calcium pantothenate. He observed a rise in the level in 4-8 h and a decrease in 12 h after the administration.

Pantothenic acid content in human milk and assay methods reported in the literature are summarized in Table 2.

Reference	Type of Milk	PA/ml (microgram)	Enz.Trt	Assay
Johnston et al. (1981)	Mature:fore hind:	6.7(5.8-7.6) 6.7(1.9-15.2	Intestinal)phos.+pigeon liver enz.	Micro- L.plantarum
Dept. of Hth & Social Security (1977); England	Mature	2.6(2.2-3.3)	Alk.phos.+ pigeon liver extract	Micro- L.plantarum
Srinivasan & Belavady (1976);India	Colostrum Transitiona Mature	1.3 [±] 0.3 12.2 [±] 0.2 2.3 [±] 0.4	None None None	Micro- L.plantarum
Telegdy-Kovåts & Szorady(1968); Hungary	Mature	(1.9-2.5)	Papain+ takadiastas	Micro- Saccharomyces clisbergensis
Davidov & Kruglova(1961); USSR	Mature	1.4(0.7-2.7)		Micro-
Deodhar & Ramakrishnan (1960);India	Mature	1.4(1.0-1.8)	Intestinal phos.+chix. liver enz.	Micro- L.arabinosus
Karlin R.(1957); France	Mature	2.1(1.1-4.5)	Pancreatin; papain+ takadiastase	Micro- L.casei
Pellegrini & Chiari(1955); France	Colostrum (Mature	(1.2-1.4) 2.5(2.0-3.6)	Papain+ takadiastase	Micro- L.arabinosus

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Table 2. Pantothenic acid content of human milk reported in the literature.

Table 2. Continued

Reference	Type of Milk	PA/ml (microgram)	Enz.Trt	Assay
Pratt et al. (1951)	Mature	2.5(1.6-3.0)	None	Micro- L.casei
Schmidt(1950)	Mature	2.2(1.7-3.0)		Micro- Streptomyces plantarum
Macy(1949)	Colostrum Transitional Mature	1.8(0.3-3.0) 2.9(1.4-4.1) 2.5(0.9-5.8)	None None None	Micro- L.casei
Coryell et al. (1945)	Colstrum (1st day) (4th day) Transitional Mature	0.5 2.5 3.0 2.5(0.9-5.8)	None	Micro- L.casei
Williams et al. (1942)	Mature	1.6(0.8-3.0)	Autolysis; autoclave; papain+ takadiastase	Micro- L.casei

Numbers in parenthesis represent ranges. Mean[±]standard error of mean enz=enzyme phos.=phosphatase L.=Lactobacillus Micro=microbiological assay

Interpretation and comparison of data on milk composition requires special attention to the different methodologies used as summarized in Table 2. For determination of pantothenic acid in milk Pellegrini and Chiari (1955) used microbiological assay without prior enzyme treatment and with a combination of papain and takadiastase. They found that the assays with prior enzymic hydrolysis gave a slightly higher mean value (2.54 microgram/ml) than that without (2.06 microgram/ml). Karlin (1957) compared three different methods of sample preparation: no treatment, with pancreatin treatment and with a combination of takadiastase and papain. The author also observed increased liberation after enzyme treatments, no difference between the two enzymic hydrolysis and very large individual variation (1.10 -4.50 microgram/ml). These observations are inconclusive because they lack statistical analysis, particularly considering the large individual variation. Williams et al. (1942) investigated the effectiveness of enzymic liberation of pantothenic acid from milk in three different ways: 24 h autolysis, autoclaving for 30 min. at 15 pounds pressure and hydrolysis with takadiastase and papain. The mean value of the three different methods were 2.6, 2.5, and 2.9 microgram/ml respectively. The authors indicated that the extra pantothenic acid yield observed after enzyme digestion was not sufficient to account for the vitamin added with the enzymes. Validation of the various methodologies along with identification of the forms of the vitamin in milk remain for further research. In
general, studies support the conclusion that the majority, if not all, of the vitamin in milk is in readily absorbable free form which does not require enzymic hydrolysis prior to the assays.

<u>Relating Blood, Urine and Dietary</u> Levels of Pantothenic Acid

Levels of blood or urinary excretion were related to the dietary intake and nutritional status in several surveys. Data indicated that average daily intakes from American mixed diets ranged from 2.9 mg to 13 mg per day and that variation among individuals was very large. Ahrens and Boucher (1978) estimated the mean content of pantothenic acid in the "USA diet" homogenate as 45 mg per day. They analyzed by the microbiological method the vitamin content of a hypothetically simulated American diet which could be different from actual consumption. Chung et al. (1961) analyzed pantothenate content in planned "high cost", "low cost" and "poor" American diets and reported average daily intakes of 16.3 mg, 14.2 mg and 6 mg respectively. They concluded that the total pantothenic acid activity of the adult diet may be expected to vary from 10 to 20 mg daily. Urinary excretion is correlated with dietary intake among individuals and among published data as seen in Figure 1.

In an attempt to examine the relationship between diet and urinary excretion, Fox and Linkswiler (1961) provided three different



Fig 1. Correlation between urinary excretion and dietary intake among the published data.

levels of pantothenate to eight women. Mean excretions following daily intakes of 2.8 mg, 7.8 mg and 12.8 mg of pantothenic acid were 3.2 mg, 4.5 mg and 5.6 mg respectively and resulted a significant regression between the two variables (r=0.805). The results also indicated that low daily intake of 2.8 mg pantothenic acid allowed negative retention while higher intakes allowed large positive retention. This negative balance of the vitamin was also observed in another study (Fry et al., 1976) in which male subjects fed no pantothenic acid for ten weeks excreted 3.05 mg during the first week and 0.75 mg during the 10 weeks.

Schmidt (1951) examined urinary excretion of pantothenic acid in young and old individuals and observed moderately lower excretion in the elderly subjects under normal condition and following parentral pantothenic acid administration. This study did not estimate dietary intake but the author suspected lower excretion might be due to decreased food intake.

A few studies attempted to examine the interaction between the pantothenic acid and other nutrients based on the urinary excretion. Koyanagi et al. (1969) reported increased urinary excretion of pantothenic acid after thiamin administration. The effect was not observed after the administration of riboflavin, pyridoxine, ascorbic acid, nicotinic acid, vitamins A and B_{12} . positive interaction was observed between the dietary protein level and urinary pantothenic acid excretion in rats (Tao and Fox, 1976^a). Duke et al. (1977) reported an increased niacin excretion upon pantothenic acid

supplementation, a depressed pantothenic acid excretion upon niacin supplementation and increased pantothenic acid excretion upon methionine supplementation. The authors indicated some possible dangers in indiscriminate supplementation of food products.

In an effort to elucidate the metabolism and excretion mechanism of pantothenic acid, a few studies focused on urinary pantothenic acid excretion in conjunction with blood levels. Roholt and Schmidt (1951) observed that in man an active secretion of pantothenic acid took place in the tubules as soon as the plasma concentration exceeded about 0.5 microgram/ml. At very low plasma concentration active resorption occurred in the tubules and the body tried to retain the vitamin as long as it was in physiological concentration. In human beings with normal glomerular filtration rates, an extra urinary excretion of free pantothenic acid was observed after intravenous administration of CoA and calcium pantothenate (Bigler and Tholen, 1966). The urinary excretion of pantothenate corresponded to 20-30%and 15-30% of the dose infused in the forms of CoA and calcium pantothenate respectively. The percentage of the recovered vitamin in urine was higher (42%) with a similar dose of calcium pantothenic acid infusion in a study by Baker et al. (1969).

For evaluation purposes, urinary excretion of less than 1.0 mg/day was suggested by Sauberlich (1980) as abnormally low for a human adult. For urine data the variation contributed by the methodology is thought to be smaller because urine is reported to contain only free pantothenic acid. Thus the step of enzymic

Level ng/ml	Method of Assay	Enzyme Treatment	Subjects	Author
533 ± 22.5 ^a	RIA	Alk phos plus pantetheinase	37 noninstitu- tionalized elderly	Srinivasan et al. (1981)
583 ± 51.9 ^a	RIA	Alk phos plus pantetheinase	l7 institution- alized elderly	Srinivasan et al. (1981)
890 ± 240 ^a	microbiological - Lactobacillus plantarum	Alk phos plus chicken liver enz	9 women taking oral contraceptive agent	Lewis and King (1980)
1270 ± 170 ^a	microbiological - Lactobacillus plantarum	Alk phos plus chicken liver enz	4 women, 19-25 years of age	Lewis and King (1980)
1700 ± 90 ^b 50 - free 1600 - bound	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	29 chronic ulcerative and granulomatous colitic patients	Ellestad- Sayed et al. (1976)
570 ± 2.6 ^b	microbiological - Lactobacillus plantarum	Alk phos plus hog kidney extrac	23 pregnant women t	Srinivasan and Belavady (1976)
634 ± 80.6^{b}	microbiological - Lactobacillus plantarum	Alk phos plus hog kidney extrac	12 lactating women t	Srinivasan and Belevady (1976)

Table 3. Mean pantothenic acid levels in human blood reported in the literature.

Table 3. (continued)

Level ng/ml	Method of Assay	Enzyme Treatment	Subjects	Author
626 ± 55.3 ^b	microbiological - Lactobacillus plantarum	Alk phos plus hog kidney extract	14 nonpregnant	Srinivasan and Belavady (1976)
883 ^e	microbiological	Alk phos plus pigeon liver enz	l8 surgical patients	Ellestad-Sayed et al. (1976)
250 (200-800) ^C	microbiological - Tetrahymena pyriformis	Clarase	76 nonpregnant healthy females	Baker et al. (1975)
438 (228 - 680) ^d	microbiological - Tetrahymena pyriformis	Clarase	174 mothers at parturition	Baker et al. (1975)
1830 ± 600 ^a (1050 - 2420) ^c	microbiological - Lactobacillus plantarum	Alk phos plus chicken liver enz	4 nonpregnant girls	Cohenour and Calloway (1972)
1030 ± 260 ^a (530 - 1390) ^c	microbiological - Lactobacillus plantarum	Alk phos plus chicken liver enz	18 pregnant teen- agers, ages 14-18	Cohenour and Calloway (1972)
1064 (966 - 1156) ^d 97 - free 964 - bound	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	39 women, ages 40-44	Ishiguro (1972)
290 (145 - 580) ^d	microbiological	Clarase	642 NYC school children, ages 10–13	Baker et al. (1967)

Table 3. (continued)

Level ng/ml	Method of Assay	Enzyme Treatment	Subjects	Author
130 - 500	microbiological - Tetrahymena pyriformis	Unknown	30 healthy hospita and laboratory personnel	l Leevy et al. (1965)
1077 ± 83.2 ^C	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	20 non-vegetarian	Barton-Wright and Elliot (1963)
2622 ± 115.0 ^C	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	9 vegetarian	Barton-Wright and Elliot (1963)
617 (505 - 728) ^C 100 - free 517 - bound	microbiological - Tetrahymena pyriformis	Alk phos plus pigeon liver enz	pregnant women in 4th to 5th month o pregnancy 20-30 yrs. of age	Ishiguro f (1962)
966 (626 - 1306) ^d 118 - free 848 - bound	microbiological - Tetrahymena pyriformis	Alk phos plus pigeon liver enz	5 women	Ishiguro (1962)
464	microbiological - Lactobacillus plantarum	Alk phos plus of both sexes, ages 15-60	30 healthy persons	Hatano (1962)

Table 3. (continued)

Level ng/ml	Method of Assay	Enzyme Treatment	Subjects	Author
470 (110-1600) ^C	microbiological - Tetrahymena pyriformis	Clarase	367 normal subjects	Baker et al. (1960)
560 (220-1900) ^C	microbiological - Lactobacillus plantarum	Clarase	367 normal subjects	Baker et al. (1960)

a_{mean ±} SD bmean ± SE cnumbers in parentheses denote range dnumbers in parentheses denote 95% confidence limits evalues are computed from red blood cell levels assuming a hematocrit of 45

Level	Method of Assay	Enzyme Treatment	Subjects	Author
196.7 ± 37.7 ^b	microbiological - Lactobacillus plantarum	Alk phos plus hog kidney enz	9 nonpregnant	Srinivasan and Belavady (1976)
169.2 ± 19.0 ^b	microbiological - <u>Lactobacillus</u> plantarum	Alk phos plus hog kidney enz	16 pregnant	Srinivasan and Belavady (1976)
193.1 ± 24.5 ^b	microbiological - Lactobacillus plantarum	Alk phos plus hog kidney enz	8 lactating women	Srinivasan and Belavady (1976)
130 ± 10 ^b 50 free 80 bound	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	chronic ulcerative and granulomatous colitic patients	Ellestad -Sayed et al. (1976)
3840 ± 1960 ^a	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	76 individuals of different ages and sex	Markkanen (1973)
200 (152-400) ^C	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	representative population	Baker and Frank (1968)

Table 4. Mean pantothenic acid levels in human plasma or sera reported in the literature.

Table 4. (continued)

	Level	Method of Assay	Enzyme Treatment	Subjects	Author
160 ± 21 ^a	÷	microbiological - Lactobacillus plantarum	Alk phos plus pigeon liver enz	20 villagers in Japan	Koyanagi et al. (1967)

amean ± SD bmean ± SE cnumbers in parenthese denote range dnumbers in parentheses denote 95% confidence limits evalues are computed from red blood cell levels assuming a hematocrit of 45

hydrolysis is eliminated.

Blood contains relatively high levels of the vitamin but whether changes in the level can indicate a pantothenic acid deficiency is uncertain. Few studies have attempted to correlate the pantothenate levels in blood and dietary intake and those which did found poor correlations. In a metabolic study with 10 adult males fed a pantothenic acid deficient diet (Fry et al., 1976), blood pantothenic acid levels slightly decreased from 1950 ng/ml at the beginning to 1520 ng/ml after 10 weeks. However the control group, whose diet was supplemented with 10 mg per day, showed no significant change in their blood levels. Feeding as much as 100 mg pantothenic acid daily increased the blood level in both groups of subjects to 2700 ng/ml for the period and no toxicity was observed.

Pantothenic acid levels in human blood and plasma reported in the literature are summarized in Table 3 and Table 4. The levels in blood reported in the literature range from 59 ng/ml to 2622 ng/ml for whole blood and that of serum range from 137 ng/ml to 1830 ng/ml serum. Because of these wide ranges in reported data, interpretation of data differs among researchers. Sauberlich et al. (1974) indicated a total pantothenic acid level of less than 1000 ng/ml of whole blood was suggestive of low or inadequate dietary intake of the vitamin. However, Baker and Frank (1968) and Baker et al. (1975) suggested that total pantothenic acid levels of less than 160 ng/ml of whole blood represent hypovitaminosis. The published data of Baker's laboratory have been consistently lower than those of many others. The forms and distribution of the vitamin in different blood compartments are of interest. Blood contains both free and a bound form of pantothenate. Of these, most of the bound pantothenic acid exists in the erythrocytes whereas a small amount of free pantothenate is present in plasma (Baker et al., 1969).

To examine the vitamin distribution in body fluid, Baker et al. (1969) injected multivitamins containing 45 mg D-panthenol intravenously into seven adult males. They observed the peak plasma pantothenic acid level after 3 min of injection with no changes in erythrocyte content. A gradual decrease in plasma level and increase in red cell concentration were observed for 24 h. Yet at peak plasma concentration, 96% of the exogenous vitamin was present within tissue pools and only 4% was found in both plasma and erythrocytes. These data suggest that pantothenic acid level in plasma may be a very short-term indicator of nutritional status and may be fairly constant in fasting blood samples. Red blood cell level or whole blood content, instead, appears to be more responsive to dietary changes or status. Similar results were obtained when 250 micromoles of CoA and 250 micromoles of D-pantothenic acid were injected into convalescent patients with normal glomerular filtration rates (Bigler and Tholen, 1966). In the study blood levels of free and bound pantothenic acid were elevated during and 20 h after the infusion and the increase was mostly due to the bound pantothenic acid.

In general, urinary pantothenic acid value appear to be a more sensitive indicator of intake than do blood or plasma pantothenic acid levels (Sauberlich et al., 1974; Srinivasan et al., 1981; Fox and Linkswiler, 1961; Fry et al., 1976).

Pantothenic Acid Excretion per Creatinine

In an effort to standardize variations in urinary excretion and collection, many researchers report data as a ratio of a particular nutrient in the urine to urinary creatinine. The rationale for this approach is that the daily urinary excretion of creatinine is relatively constant which permits extrapolation of a random specimen to 24 h basis. This assumption is based on a classical studies of Folin (1905).

Many investigators have since reported that the quantity of daily urinary creatinine in humans and in animals is significantly correlated with lean body mass and/or muscle mass (Chinn, 1967; Van Nierkerk et al., 1963; Muldowney et al., 1957; Ryan et al., 1957). However, it has been shown in past years, that daily creatinine excretion can vary widely even under highly controlled conditions of collection (Greenblatt et al., 1976), dietary intake (Crim et al., 1975) and physical activity (Refusum and Stromme, 1975). Lis et al. (1972) reported that excretion of creatinine in the same individuals with controlled activity and food intake can vary 3-4 fold from voiding to voiding within a same day and from day to day. They proposed nocturnal-diurnal variation in creatinine excretion

associated with muscle cell recovery. Calloway and Morgan (1971) observed a gradual decline in the creatinine excretion of healthy men fed a creatine free formula diet for 88 days. The decline in creatinine excretion did not correlate with a systemic alteration of body composition measured by nitrogen and potassium balance, body density, or total body water. Similar results were observed by Bleiler and Schedl (1962). A gradual decline in creatinine excretion occurred after subjects were changed from diets containing meat to creatinine-free formula diets. In some studies, feeding creatinine to human resulted in increased urinary creatinine excretion to values 20-30% higher than those prior to creatinine feeding (Chanutin, 1926; Rose et al., 1928; Hyde, 1942; Crim et al., 1975). Creatinine excretion increased in some subjects fed precursor amino acids, arginine and glycine (Crim et al., 1975). In all of the creatine feeding studies, the diet was restricted to meat-free, creatinine-free diet or excluded creatinine containing foods and exercise was maintained constantly. Some investigators discovered that quantitative assessment of urinary creatinine depended on pH of the medium (Cooper and Biggs, 1961). They proposed that non-enzymic acid catalyzed conversion of creatine to creatinine was a lactate dependent mechanism. Although creatine excretion occurs more regularly in young children than in adults, and in women than in men, the total creatinine excretion may vary depending on the conversion of the creatine to creatinine in acid urine.

CHAPTER III

MATERIALS AND PROCEDURES

Subjects

The number of subjects needed for this study was determined to obtain a 95% confidence interval of not more than 60 ng pantothenic acid per ml of whole blood based on the published data from our lab and preliminary work. Ten subjects was suggested for each group from the calculation. Since the pregnant women at the beginning were to be divided into a nursing and a bottle-feeding groups at postpartum, twice the number was assigned for the experimental group than those for the non-pregnant control group. Expecting a large number of drop-outs during the 6-7 months follow-up study period, a twice the suggested number i.e., 20 was chosen as the subject number for recruitment for each group. Thus 40 pregnant women and 20 non-pregnant women were voluntarily involved at the beginning.

In an effort to reduce the large individual variation reported in the literature, a group of middle class, 20 to 35 years of age, Caucasian women was selected locally. The majority of the pregnant subjects was recruited through cost-involved prenatal classes offered by a local hospital and La Maz classes. A small number of the participants was selected through local obstetricians' referrals and a local newspaper advertisement. No subject in this study was a recipient of governmental social programs such as the Women, Infants and Children program. The longitudinal study was designed also to reduce the risk of large individual variations masking the group effect.

At the time of recruitment, all the pregnant women were between 26-30 weeks of gestation. Planned feeding method for the neonate was not one of the selection criteria for the subjects. Each woman in the experimental group was encouraged to make her own decision on feeding method for her infant. The control group was composed of 20 non-pregnant, non-lactating Caucasian women who had demographic backgrounds comparable with those of the experimental group. Both groups of women were free of known chronic diseases such as diabetes mellitus or hypertension and were taking no medication except nutritional supplements and oral contraceptives.

All subjects completed the informed consent form (Appendix A) at the first interview with the researcher when the purpose and procedure of the study and extent of the participants' involvement were explained. The research protocol was approved by the Human Research Committee of Utah State University (Appendix B). All subjects provided basic demographic information including age, height, weight, number of children, use of oral contraceptives (previous use for the pregnant and present use for the control group), gestational age, expecting date of birth, planned feeding method for neonate etc. (Appendix C). A thorough individual instructions on sample collection and diet record was presented to each participant prior to beginning the study. Food models, household measuring devices and a completed example dietary record were used to assist in the completion of the dietary record. (Appendix D).

Sample Collection

Subjects were evaluated for their pantothenic acid status at the third trimester of pregnancy (30 to 32 weeks gestation, period I), at two weeks postpartum (period II) and at 3 months postpartum (period III). The women in the control group followed the same schedule for their participation.

Each subject completed a two-day dietary record and donated a fasting blood sample for each period. The dietary intake record was re-examined and cross-checked to ensure correct measurements and recording with 24 to 48 h recalls in the following morning when the blood sample was obtained. This method was reported as highly reliable in the pregnant population by Rush and Kristal (1982). The fasting blood sample was drawn by venipuncture into a heparinized vacuum tube in the morning (5:00 AM to 9:30 AM) at each subject's residence by a registered medical technologist. The blood sample was kept in ice for transportation to the laboratory where it was divided into two aliquots: one for whole blood and the other for plasma. These were kept frozen until analyzed.

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On the second day of the diet record at period II and period III, minimum 10 mls each of fore and hind milk samples were separately collected in sterilized plastic containers at the first feeding between 6:00 AM and 9:00 AM by the nursing subjects. Upon collection all samples were kept frozen until transported to the laboratory and analyzed.

Two 24 h urine samples were collected by all subjects in plastic jugs which were provided and contained an antimicrobial agents, thimerosal, for the same two days of the diet record at periods I and III. Urine samples were not collected at period II because of the possibility of blood contaminated samples from the experimental group. The 24 h urine samples were measured for volume and creatinine and a portion was frozen for pantothenate analysis.

Chemical Anaysis

Sample Preparation

Blood samples, 0.5 ml, were hemolyzed by three quick freeze/thaw cycles and subjected to a double enzyme treatment with 5 units (one unit hydrolyzes 1.0 micromole of p-nitrophenylphosphate to p-nitrophenol and inorganic phosphate per min at pH 10.4 at 37 C) bovine intestinal alkaline phosphatase (Sigma Chemical Co., P5521), 0.1 unit (one unit hydrolyzes 1.0 micromole of pantethein per min under the condition of the mercaptide assay; Wittwer et al., 1982)) of pantetheinase (purified by Wittwer et al., 1982) and 0.4 ml of 0.1 M Tris(hydroxymethyl)aminomethane (Tris) buffer (pH 8.1) in a total volume adjusted to 1 ml with distilled water. After 7-8 h of incubation at 37 C in a shaker bath, the hydrolysis was terminated by addition of saturated $Ba(OH)_2$ and 10% Zn(SO)₄ in equimolar concentrations and centrifugation at 4000xg for 10 min. The resulting protein-free clear supernatant was used for pantothenate analysis by RIA (Wyse et al., 1979).

The equimolar ratio of saturated $Ba(OH)_2$ and $10\% Zn(SO)_4$ was determined by titrating 10 ml of $10\% ZnSO_4$ diluted with an equal volume of distilled water against saturated $Ba(OH)_2$ with phenolphthalein as an indicator. To prepare the saturated $Ba(OH)_2$ an excess amount was dissolved in warm water with continous stirring overnight. The resulting solution was quickly vacuum filtered using Whatman No.1 filter paper and kept tightly capped. Approximately 0.5 ml of each solution was used for complete deproteinization of 0.5 ml biological fluids. Unequal molar concentrations of the reagents to samples resulted in incomplete precipitation.

Plasma, 0.5 ml, diluted with an equal volume of distilled water was deproteinized by addition of saturated Ba(OH)₂ and 10% ZnSO₄ in equimolar concentration and followed by centrifugation (4000xg, 10 min). The resulting clear colorless aliquot was used for pantothenic acid determination by the RIA.

Contrary to the published data (Markkanen, 1973; Ellestad-Sayed et al., 1976) and the common use of enzyme treatments for sera or plasma (Table 3), no bound form of pantothenate was detected by

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preliminary studies. When twenty plasma samples were assayed with and without the double enzyme treatment, no significant difference was found between the two treatment (Table 5). In our assay a pantetheinase (Wittwer et al., 1982) containing a trace amount of endogenous pantothenate was used instead of the traditional non-specific crude commercial avian liver enzymes containing appreciable amount of pantothenate.

Human milk, 0.5 ml, was incubated with 5 units of bovine intestinal alkaline phosphatase (Sigma Chemical Co., p5521) and 0.4 unit pantetheinase (purified by Wittwer et al., 1982) in a total volume of 0.5 ml with 0.01 M Tris buffer (PH 8.1) at 37 C for 7-8 h.

Table 5. Plasma pantothenic acid levels determined after with/without enzyme treatment.

Description	No enzyme	Enzymesa	t-Test
Mean(ng/ml)	96.85±8.41	96.45±8.05	ns
Range(ng/ml)	48 -170	52 -180	
Recovery(%)	112.9	99.4	ns

Mean±standard error of mean a=alkaline phosphatase (5 units) plus pantetheinase (0.1 unit) per 0.5 ml milk

The hydrolysis was terminated by addition of saturated $Ba(OH)_2$ and 10% $ZnSO_4$ in eqimolar concentration. The deproteinized clear aliquot obtained after centrifugation (6000xg, 20 min) was used for the RIA (Wyse et al., 1979).

To validate the above methodology, the three following hypotheses were tested before assaying samples: a) milk fat globules do not entrap pantothenic acid; b) enzymic hydrolysis of the sample does not free a bound form of pantothenic acid existing in milk; and c) chemical deproteinization technique produces the same result as dialysis, boiling or autoclaving procedures.

To test the first hypothesis, sonication and homogenization were used to disperse the fat molecules in milk and to release the hypothetically entraped pantothenic acid from the molecules. The completion of homogenization was evaluated by examining the fat molecules under a microscope (1:10 dilution, 100X) after treatments. The sonication produced heterogeneous fat molecule sizes while homogenization produced homogeneous small fat molecules. The second hypothesis was evaluated by subjecting human milk samples with or without the double enzyme treatment with alkaline phosphatase (5 units) and pantetheinase (0.4 unit) per 0.5 ml of the milk sample. The third hypothesis was tested by treating the samples with the following different deproteinization techniques: a) chemical deproteinization with saturated $Ba(OH)_2$ and 10% ZnSO4; b) dialysis using dialyzing tubes over night; c) boiling (100 C, 2 min); and d) autoclaving (100 C, 30 min). These results are summarized in Table 6.

The t-tests failed to reject the null hypotheses that a) milk fat contains no extra pantothenic acid and c) the chemical deproteinzation and dialysis produces the same results. The hypothesis that enzymic treatment does not produce increased pantothenic acid was rejected at the level of p=0.05. The increment in pantothenic acid value due to the enzymic hydrolysis was 13.7% of the value obtained without enzyme

treatment. Milk samples boiled or autoclaved produced an opaque solutions after centrifugation (4000 xg for 20 min). A dilution (1:10) of the supernatant had an absorbance of 2.82 at 280nm. The aliquot interfered with the RIA procedure by producing a large pellet

Но	Description of milk	n	Mean (mic	SD rogram/ml	t-value)	Recovery
a)	Raw	6	1.847	0.204		102%
• •	Sonicated	6	1.778	0.154	0.66(ns)	103%
D)	Raw Homogenized	6	2.425	0.300	1.64(ns)	102%
c)	No enz. With double en	10 z.10	1.810 2.045	0.200 0.282	2.15(p<0.	98% 05) 97%
d)	Chemical Dialysis Boiling Autolysis	10 10 10 10	2.100 2.250 - -	0.220 0.324	1.21(ns)	100% 104%

Table 6. Milk experiments for validation of assay technique

SD=standard deviation ns=no significance

of protein precipitate upon addition of saturated $(NH_4)_2SO_4$ in the process of separating the antibody bound pantothenic acid from free forms. The radioactivity determined by the liquid scintillation counter with the sample vial was inconsistent.

Urine samples, thawed, centrifuged (4000xg, 10 min) and diluted 1:20 with distilled water were used directly for RIA (Srinivasan et al., 1981; Wyse et al., 1979). Urinary creatinine was quantitated by the alkaline picrate method (Heinegard and Tidestrom, 1973).

Radioimmunoassay(RIA)

Total pantothenate in whole blood, plasma, human milk and urine was quantitated by the RIA deveolped by Wyse et al. (1979).

The radioimmunoassay procedures are summarized as follows: 1. Prepare sample extracts to be analyzed as described above in sample preparation. Deproteinization steps yield clear aliquots with $A_{280} < 1.0$.

2. Prepare standard solutions: Exactly 216.82 mg $Ca(PA)_2$ is dissolved in 1 l volumetric flask, adjusted to 1 l and mixed thoroughly. For appropriate dilutions of standards, to each 100 ml volumetric flask numbered 1 to 8, a volume of 1.73 ml, 0.988 ml, 0.494 ml, 0.297 ml, 0.173 ml, 0.0988 ml 0.0494 ml and 0.0247 ml of the above stock standard solution (200 ng/ml) are added respectively. Adjust the volume to 100 ml, mix thoroughly, and keep under refrigeration. The standards contain 350ng, 200 ng, 100 ng, 60 ng, 35 ng, 20 ng, 10 ng and 5 ng pantothenate per 100 microliter and can be used for at least 3 months.

3. Prepare 1% (w/v) rabbit serum albumin solution in phosphate buffer solution, add 1 ml of 1:100 dilution of thimersal per 100 ml of the 1% RSA solution and keep it at 5 C. Dilute 14 C pantothenic acid (New England New Clear, Boston, MA) to approximately 5000 cpm/200 microliter. Thaw antisera.

Pipet 0.1 ml of standard solution to each polypropylen omni-vial
(3.5ml size, Wheaton Scientific, Millville, N.J.) and diluted with 0.4

ml of distilled water. Sample extracts, 0.5 ml, are pipeted into numbered vials.

5. To each of the above add approximately 5000 cpm 14 C-pantothenic acid and 1:100 dilution of antiserum: albumin solution mixtures. (Approximately 0.5 ml per sample and standards).

6. Incubate 15 min at room temperature in shaker for competitive binding of antibody between hot and cold pantothenic acid.

7. Add 1 ml saturated $(NH_4)_2SO_4$ to each tube, mix and centrifuge at 12000xg for 12 min. Discard the supernatant.

8. Resuspend with 0.5 ml 50% saturated $(NH_4)_2SO_4$, mix and centrifuge at 12000xg for 12 min. Discard the supernatant.

9. Dissolve the resulting precipitate of antibody-bound pantothenic acid with 0.3 ml Soluene 350 (Packard Instrument Co.) and mix thoroughly.

10. Digest the above tightly capped at 60 C for 3 min.

11. Add 3.0 ml Dimilum (Packard Instrument Co.) to each vial and mix. After 20-30 min, start to read the radioactivity in liquid scintillation counter.

12. Complete a standard curve by drawing the pantothenic acid (ng) per vial versus percent binding (standard reading/blank reading x100) on probit graph paper. A straight line is produced between 10 ng to 300 ng.

13. Read the pantothenic acid content in unknown samples from the standard curve and calculate the results.

Data Analysis

Daily dietary intake of pantothenic acid and the addition of dietary supplements were determined using a USDA computer data base which was developed at Utah State University in conjunction with a contract with the Human Nutrition Information Service (HNIS) of Food and Nutrition Service (F&NS) of USDA. The data base contains 460 food items with 27 nutrients: kcal, protein, fat, carbohydrate, calcium, magnesium, phosphorus, vitamin A, thiamin, riboflavin, niacin, vitamin ^B₆, vitamin B₁₂, ascorbic acid, folacin, added sugar, total saturated fatty acid, total polyunsaturated fatty acid, total monounsaturated fatty acid, potassium, iron, sodium, zinc, cholesterol, pantothenic acid and alcohol. The major references for the pantothenic acid data were the Revised Agricultue Handbook 8 (USDA, 1976-1982), the Home Economic Research Report No.36 (Orr, 1969), the McCance and Widdowson's Food Composition (Poul and Southgate, 1978) and other published research reports (Walsh et al., 1981; Meyer et al., 1966; Zook et al., 1956). The major references for other nutrients were the Revised Agricultue Handbook 8 (USDA, 1976-1982), the USDA provisional tables, the journal articles written by USDA staff, other USDA publications, USDA data tapes and other research reports.

For complete calculation of all nutrients using the computer system for this study, the data base was expanded with composition of multivitamins, iron, vitamin C and calcium pills and other dietary supplements such as Nutrilite XX (Amway) and brewer's yeast. Pantothenic acid contents of mixed food items which were eaten by subjects and unlisted in the data base were calculated based on the ingredient information or the values for comparable foods.

The average daily dietary intake of each individual at different periods was calculated from the diet only and the diet plus dietary supplements, after the information on group number, period, subject code, code for eating occasion, food codes and amount of food consumed were entered into the computer. The data were stored in the computer and used for the statistical analysis.

Pearson's correlation coefficients were obtained and tested among demographic variables (age, height, weight, number of children etc.), biochemical variables (blood, plasma, urine, milk) and dietary intake (with and without supplements) using the Statistical Package for Social Science (SPSS). For each variable, mean, standard deviation, standard error and range were obtained. Analysis of covariance was used to evaluate statistical differences among the adjusted means of biochemical and dietary variables among the three different groups at three periods using a different statistical program (RUMMAGE). Significances in pairwise comparisons among the adjusted means were determined by the least significant difference (LSD) mean comparison tests.

Both the daily pantothenic acid excretion and the ratio of pantothenic acid per creatinine were used for the correlations and statistical analysis of urine values. For milk data the fore and hind milk values were separately analyzed and assessed. Dietary evaluation of pantothenic acid was divided into the unsupplemented group and the total population because only eleven observations of the total 138 observations were supplemented.

CHAPTER IV

RESULTS

Subjects

Eight pregnant women and three non-pregnant women withdrew their participation before the end of the study. The majority of the withdrawn population relocated during the summer of 1982.

The pregnant women at postpartum were divided into nursing and bottle-feeding groups without the influence of the researcher. Three individuals in this group switched their infant feeding method from nursing at two weeks postpartum to bottle-feeding at three months postpartum and were therefore excluded from the statistical analysis. The non-pregnant control subjects maintained their health and had no significant physiological change during the study period. The subjects who completed participation at all study periods and were included in the statistical analysis were twenty-six in the nursing group, three in the bottle-feeding group and seventeen in the control group.

Means of age, height and weight (pre-pregnant weight for the experimental group) of the subjects are summarized in Table 7. Of the twenty nine pregnant women, sixteen were primigravida, nine were secundigravida and four were multigravida. Table 7. Demographic variables of study subjects

Group	Subject (n)	Age (year)	Height (cm)	Weight (kg)	
Control	17	25.5±5.0	166.3±6.9	59.5±9.7	
Experimental	29	25.0±3.0	164.6±4.8	56.3±9.8	

Mean±standard deviation

No significant difference was observed between the two groups in demographic variables.

Dietary Intake

All Nutrients and Interactions

Dietary nutrient intakes of the control group, the pregnant women, the bottle-feeding women and the nursing women are summarized in Table 8 and Table 9. Intakes of the control group represent the average of the three periods (51 observations); the pregnant women intake is the average of all pregnant women at the gravid period (Period I, 29 observations); intake of the bottle-feeding women represent the average intake of the group at two weeks postpartum (period II) and at three months postpartum (period III) for a total of six observations; and the intake of the nursing women represent the average intake of the group at two weeks postpartum (period II) and at three months postpartum (period III) for a total of six observations; and the intake of the nursing women represent the average intake of the group at two weeks postpartum (period II) and at three months postpartum (period III) for a total of fifty-two observations. The correlations in Table 8 between pantothenic acid and other nutrients were based on the entire observations. The nutrients which had significant positive correlations with pantothenic acid intake included protein, fat, calcium, iron, magnesium, phosphorus, vitamin A, thiamin, riboflavin, niacin, vitamin B₆, vitamin B₁₂, ascorbic acid, folate, potassium and zinc. Carbohydrate, added sugar and alcohol intake had negative correlations but were not statistically significant. The mean daily calorie and protein intake of the control women, the pregnant women, the bottle-feeding women and the nursing women were 1803 kcal, 72 g; 2073 kcal, 81 g; 1725 kcal, 60 g; and 2042 kcal, 81 g, respectively. The differences among the groups were not statistically significant. Correlation coefficients and significance levels among all nutrients are shown in Appendix E.

The mean daily calorie and protein intakes of the group taking no supplements, the group taking supplements and the total subjects were 1930.09 kcal, 75.73 g; 2126.40 kcal, 91.54 g; 1945.74 kcal, 76.99 g , respectively. Statistically there was no difference among the groups. The pantothenic acid intake of the subjects taking no supplement had highly significant correlations (p<0.001) with calorie and protein intakes. When the pantothenic acid intake was divided by the calorie intake in the unsupplemented group, the ratio was 2.75 mg per 1000 kcal intake.

Pantothenic Acid Intake of All Study Population

All pregnant women and seven of the seventeen non-pregnant women in this study were taking various dietary supplements.

Observation	r ^a n=138	Control Women n=51	Pregnant Women n=29
kcal	0.09 p=0.153	1803±807 (672-6471)	2072±467 (1133-2946)
Protein (g)	0.29 p=0.000	72±23b (26-159)¢ 40 ^d (14-88) ^e 164.1% [†]	81±20 (46-127) 39 (22-61) 109.9%
Fat (g)	0.22 p=0.005	75±29 (19–181) 41 (10–100)	86±26 (37-130) 41 (18-62)
Carbohydrate (g)	-0.06 p=0.254	210±134 (46-1028) 117 (25-571)	252±58 (146–373) 121 (70–180)
Calcium (mg)	0.47 p=0.000	1093±651 (240-3720) 606 (133-2064) 136.7%	1390±750 (50-4532) 670 (313-2187) 115.8%
Magnesium (mg)	0.60 p=0.000	348±219 (126-1228) 193 (70-681) 116.2%	364±119 (172-688) 175 (83-332) 81.0%
Phosphorus (mg)	0.57 p=0.000	1387±601 (484-3327) 770 (268-1846) 173.5%	1522±486 (861-3165) 734 (415-1527) 126.8%

Table 8. Nutritional intake of all nutrients from the foods and the dietary supplements of the control and the pregnant women.

Table 8. Continue	ed	
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Observationn=138n=51n=29Vitamin A (IU)0.4811128±1013310197±5894 $p=0.000$ (902-47812)48-27901 6176 4921(500-26536)(1326-13466)278.2%203.9%Thiamin (mg)0.883.1±8.5 $p=0.000$ (0.3-51.2)(0.8-5.5) 1.7 1.2(0.2-28.4)(0.4-2.7)290.9%170.0%Riboflavin (mg)0.823.6±7.8 $p=0.000$ (0.5-48.2)(1.2-7.3) 2.0 1.6 $(0.3-26.8)$ (0.6-3.5)298.3%209.4%Niacin (mg)0.7280±202 $p=0.000$ (7-1191) $(12-340)$ 44 46 $(4-26)$ (6-164) 574.4% 607.5%Pyridoxine (mg)0.79 $p=0.000$ (0.7-46.9) $(0.4-26.1)$ (0.4-6.4) 167.5% 143.1%Vitamin B ₁₂ 0.34 $(microgram)$ $p=0.000$ $(0.5-27.5)$ (1.1-11.2) 224.0% 233.3%Ascorbic Acid0.65 $205±248$ 237 ± 91 (mo) $p=0.000$ $(15-1526)$ $(15-482)$ $(15-1526)$ $(15-482)$		rd	Control Women	Pregnant Women
Vitamin A (IU) 0.48 11128 ± 10133 10197 ± 5894 $p=0.000$ $(902-47812)$ $48-27901$ 6176 4921 $(500-26536)$ $(1326-13466)$ 278.2% 203.9% Thiamin (mg) 0.88 3.1 ± 8.5 $p=0.000$ $(0.3-51.2)$ $(0.8-5.5)$ 1.7 1.2 $(0.2-28.4)$ $(0.4-2.7)$ 290.9% 170.0% Riboflavin (mg) 0.82 3.6 ± 7.8 $p=0.000$ $(0.5-48.2)$ $(1.2-7.3)$ 2.0 1.6 $(0.3-26.8)$ $(0.6-3.5)$ 298.3% 209.4% Niacin (mg) 0.72 80 ± 202 $p=0.000$ $(7-1191)$ $(12-340)$ 44 46 $(4-26)$ $(6-164)$ 574.4% 607.5% Pyridoxine (mg) 0.79 $p=0.000$ $(0.7-46.9)$ $(0.4-26.1)$ $(0.4-6.4)$ 167.5% 143.1% Vitamin B_{12} 0.34 $(microgram)$ $p=0.000$ $(15-1526)$ $(11-11.2)$ 224.0% 233.3%	Observation	n=138	n=51	n=29
Thiamin (mg) 0.88 $p=0.000$ $\begin{pmatrix} 0.3-51.2 \\ 0.3-51.2 \\ 1.7 \\ 0.2-28.4 \\ 290.9\% \end{pmatrix}$ $\begin{pmatrix} 0.4-2.7 \\ 1.2 \\ (0.4-2.7) \\ 290.9\% \end{pmatrix}$ Riboflavin (mg) 0.82 $p=0.000$ $\begin{pmatrix} 3.6^{\pm}7.8 \\ 0.5-48.2 \\ 0.5-48.2 \\ 2.0 \\ 0.6-3.5 \\ 298.3\% \end{pmatrix}$ $\begin{pmatrix} 0.6-3.5 \\ 298.3\% \\ 209.4\% \end{pmatrix}$ Niacin (mg) $\begin{pmatrix} 0.72 \\ p=0.000 \\ 44 \\ 4-26 \\ 574.4\% \end{pmatrix}$ $\begin{pmatrix} 0.7\pm0.2 \\ 6-164 \\ 574.4\% \\ 607.5\% \end{pmatrix}$ Pyridoxine (mg) $\begin{pmatrix} 0.79 \\ p=0.000 \\ 1.8 \\ 1.2 \\ 1.8 \\ 0.4-26.1 \\ 167.5\% \end{pmatrix}$ $\begin{pmatrix} 3.7^{\pm}2.9 \\ 0.9-13.2 \\ 1.8 \\ $	Vitamin A (IU)	0.48 p=0.000	11128±10133 (902-47812) 6176 (500-26536) 278.2%	10197±5894 48-27901 4921 (1326-13466) 203.9%
Riboflavin (mg) 0.82 $p=0.000$ $3.6^{\pm}7.8$ $(0.5-48.2)$ 2.0 $(1.2-7.3)$ 2.0 $(0.6-3.5)$ 298.3% $3.4^{\pm}1.5$ $(1.2-7.3)$ 2.0 $(0.6-3.5)$ 298.3% Niacin (mg) 0.72 $p=0.000$ $80^{\pm}202$ $(7-1191)$ 44 46 $(4-26)$ 574.4% $97^{\pm}107$ $(6-164)$ 574.4% Pyridoxine (mg) 0.79 $p=0.000$ $3.3^{\pm}7.6$ $(0.7-46.9)$ 1.8 $(0.4-26.1)$ 167.5% $3.7^{\pm}2.9$ 1.8 $(0.4-6.4)$ 167.5% Vitamin B_{12} (microgram) 0.34 $p=0.000$ $6.7^{\pm}10.5$ $(0.6-49.6)$ 3.7 23.3% Vitamin B_{12} (microgram) 0.34 $p=0.000$ $6.7^{\pm}10.5$ $(0.6-49.6)$ 3.7 23.3% Ascorbic Acid (mg) 0.65 $205^{\pm}248$ $(15-1526)$ $237^{\pm}91$ $(55-429)$	Thiamin (mg)	0.88 p=0.000	3.1±8.5 (0.3-51.2) 1.7 (0.2-28.4) 290.9%	2.5 [±] 1.3 (0.8-5.5) 1.2 (0.4-2.7) 170.0%
Niacin (mg) 0.72 $g0^{\pm}202$ $97^{\pm}107$ p=0.000 $(7-1191)$ $(12-340)$ 44 4 (4-26) $(6-164)574.4% 607.5\%Pyridoxine (mg) 0.79 3.3^{\pm}7.6 3.7^{\pm}2.9p=0.000 (0.7-46.9) (0.9-13.2)1.8$ $1.8(0.4-26.1)$ $(0.4-6.4)167.5%$ $143.1%Vitamin B12 0.34 6.7^{\pm}10.5 9.3^{\pm}6.9(microgram) p=0.000 (0.6-49.6) (2.3-23.3)3.7$ $4.5(0.3-27.5)$ $(1.1-11.2)224.0%$ $233.3%Ascorbic Acid 0.65 205^{\pm}248 237^{\pm}91$	Riboflavin (mg) 0.82 p=0.000	3.6 [±] 7.8 (0.5-48.2) 2.0 (0.3-26.8) 298.3%	3.4 [±] 1.5 (1.2-7.3) 1.6 (0.6-3.5) 209.4%
Pyridoxine (mg) 0.79 $p=0.000$ 3.3 ± 7.6 $(0.7-46.9)$ 1.8 $(0.9-13.2)$ 1.8 $(0.4-6.4)$ 167.5% 3.7 ± 2.9 $(0.9-13.2)$ 1.8 $(0.4-6.4)$ 143.1% Vitamin B12 (microgram) 0.34 $p=0.000$ 6.7 ± 10.5 $(0.6-49.6)$ 3.7 $(2.3-23.3)$ 3.7 4.5 $(1.1-11.2)$ 224.0% 9.3 ± 6.9 $(2.3-23.3)$ 3.7 4.5 $(1.1-11.2)$ 233.3% Ascorbic Acid (mg) 0.65 $p=0.000$ 205 ± 248 $(15-1526)$ 237 ± 91 $(55-438)$	Niacin (mg)	0.72 p=0.000	80±202 (7-1191) 44 (4-26) 574.4%	97±107 (12-340) 46 (6-164) 607.5%
Vitamin B_{12} (microgram)0.34 p=0.000 $6.7^{\pm}10.5$ (0.6-49.6) 3.7 (0.3-27.5) 224.0% $9.3^{\pm}6.9$ (2.3-23.3) 4.5 (1.1-11.2) 233.3%Ascorbic Acid (mg)0.65 p=0.000 $205^{\pm}248$ (15-1526) $237^{\pm}91$ (55-438)	Pyridoxine (mg)) 0.79 p=0.000	3.3±7.6 (0.7-46.9) 1.8 (0.4-26.1) 167.5%	3.7±2.9 (0.9-13.2) 1.8 (0.4-6.4) 143.1%
Ascorbic Acid 0.65 205 ± 248 237 ± 91 (mg) p=0.000 (15-1526) (55-438)	Vitamin B ₁₂ (microgrām)	0.34 p=0.000	6.7 [±] 10.5 (0.6-49.6) 3.7 (0.3-27.5) 224.0%	9.3±6.9 (2.3-23.3) 4.5 (1.1-11.2) 233.3%
(mg) p=0.000 (13-1320) (33-438) 113 114 (8-847) (27-211) 341.8% 297.4%	Ascorbic Acid (mg)	0.65 p=0.000	205±248 (15-1526) 113 (8-847) 341.8%	237±91 (55-438) 114 (27-211) 297.4%

	rd	Control Women	Pregnant Women
Observation	n=138	n=51	n=29
Folic Acid (microgram)	0.61 p=0.000	423±493 (85-2649) 234 (47-1470) 105.8%	703±487 (166–1519) 339 (80–733) 88.0%
Added Sugar (g)	-0.12 p=0.076	40±28 (1-100) 22 (1-55)	64±37 (20-154) 31 (10-74)
Total Saturated Fatty Acid (g)	0.20 p=0.01	26±11 (7-73) 14 (4-41)	30±9 (13-47) 14 (6-22)
Total Poly- Unsaturated Fatty Acid (g)	0.03 p=0.39	13 [±] 7 (2–43) 7 (1–24)	15±6 (4-32) 7 (2-15)
Total Mono- Unsaturated Fatty Acid (g)	0.17 p=0.02	27±10 (9-56) 15 (5-31)	33±11 (14-56) 16 (7-27)
Potassium (mg)	0.41 p=0.00	2831±1716 (921-9305) 1571 (511-5164)	3206±1020 (1756-5175) 1547 (847-2498)
Iron (mg)	0.27 p=0.00	18±14 (4-74) 10 (2-41) 103.1%	33±28 (7-95) 15 (3-46) 69.0%
Sodium (mg)	0.07 p=0.20	2441±1099 (820-6988) 1354 (455-3876)	2769±1628 (1183-9999) 1331 (571-4826)

Table 8. Continued

	ra	Control Women	Pregnant Women
Observation	n=138	n=51	n= 29
Zinc (mg)	0.65 p=0.00	11±8 (3-56) 6 (2-31) 78.5%	13±5 (4-28) 6 (2-13) 65.2%
Cholesterol (g)	0.45 p=0.00	334±235 (33-1381) 185 (18-766)	410±180 (157-882) 198 (75-425)
Pantothenic Aci (mg)	d 1.00 p=0.00	9.6±19.7 (2.2-108.5) 5.3 (1.2-60.2)	5.3±1.7 (1.8-8.8) 2.5 (0.9-4.3)
Alcohol (g)	-0.07 p=0.22	5±9 (0-38) 2 (0-21)	1±2 (0-8) 0 (0-4)

Table 8. Continued

a=correlation coefficient between pantothenic acid and the nutrient intake and significance level. b=mean[±]standard deviation of daily intake c=range of the daily intake d=nutrient intake per 1000 kcal e=range of the nutrient intake per 1000 kcal f=percent of RDA for the nutrient.

Observations	Bottle-feeding Women (n=6)	Nursing Women (n=52)
kcal	1724±468 (1339-2421)	2042±615 (490-2973)
Protein (g)	60±16 ^a (45-89)b 34 ^c (26-52) ^d 135.9% ^e	81±26 (37-146) 39 (18-71) 127.0%
Fat (g)	78±21 (45-102) 45 (26-59)	92±29 (38-146) 45 (18+71)
Carbohydrate (g)	203 [±] 71 (127–293) 117 (73–170)	242±68 (122–378) 118 (60–187)
Calcium (mg)	937±747 (393-2441) 543 (228-1415) 117.1%	1257±572 (198–2764) 615 (97–1353) 104.8%
Magnesium (mg)	353±245 (204-839) 204 (118-486) 117.8%	331±109 (109-582) 162 (53-285) 73.8%
Phosphorus (mg)	1111±379 (881-1881) 644 (511-1090) 139.0%	1492±576 (377–2789) 730 (185–1366) 124.4%

Table 9. Nutritional intake of all nutrients from the foods and the dietary supplements of the nursing and the bottle-feeding women.

Observations	Bottle-feeding Women (n=6)	Nursing Women (n=52)
Vitamin A (IU)	9367±11865 (2041-32398) 5431 (1183-18785) 234.2%	9951±5198 (2093-27901) 4973 (1025-13663) 165.9%
Thiamin (mg)	2.6 [±] 2.8 (1.0-8.3) 1.5 (0.6-4.3) 238.2%	2.3±1.9 (0.5-10.2) 1.2 (0.2-5.0) 150.6%
Riboflavin (mg)	3.2 [±] 3.6 (1.0-10.3) 1.8 (0.6-6.0) 246.2%	3.0±1.5 (0.7-6.7) 1.5 (0.3-3.4) 168.9%
Niacin (mg)	197 [±] 397 (14–1005) 114 (8–582) 1412.9%	90±121 (6-588) 44 (3-291) 476.7%
Pyridoxine (mg)	3.7±4.4 (1.0-11.7) 2.1 (0.6-6.8) 188.0%	3.3 [±] 3.1 (0.4-13.8) 1.6 (0.2-6.8) 134.0%
Vitamin B ¹² (microgram)	11.5 [±] 15.3 (2.6-42.2) 6.6 (1.5-24.5) 382.7%	8.2±6.5 (1.5-28.0) 4.1 (0.7-13.9) 206.8%
Ascorbic Acid (mg)	189±169 (46-521) 109 (27-302) 316.1%	201±123 (14-616) 98 (7-301) 201.5%

Table 9. Continued
Observations	Bottle-feeding Women (n=6)	Nursing Women (n=52)
Folic Acid (microgram)	882±1358 (177-3611) 511 (103-2093) 220.6%	632±574 (77-2572) 313 (38-1275) 127.8%
Added Sugar (g)	44±19 (15-74) 25 (8-42)	61±42 (2-202) 30 (1-99)
Total Saturated Fatty Acid (g)	28±9 (15-38) 16 (8-22)	32±10 (10-53) 16 (5-26)
Total Poly- Unsaturated Fatty Acid (g)	18 [±] 9 (9-32) 10 (5-18)	15 [±] 7 (5-33) 7 (2-3)
Total Mono- Unsaturated Fatty Acid (g)	25±7 (12–36) 14 (7–21)	34±12 (8-57) 17 (4-28)
Potassium (mg)	2330±1039 (1531-4306) 1315 (888-2497)	2820±1170 (129-5583) 1398 (64-2768)
Iron (mg)	39±57 (8-152) 22 (4-88) 217.4%	29 [±] 25 (5-93) 14 (3-46) 60.8%
Sodium (mg)	2457 [±] 721 (1296–3092) 1424 (751–1793)	2319 [±] 889 (621-4675) 1135 (304-2289)

Table 9. Continued

Observations	Bottle-feeding Women (n=6)	Nursing Women (n=52)
Zinc (mg)	10±2 (8-14) 6 (5-8) 64.7%	12±6 (3-30) 6 (2-15) 49.1%
Cholesterol (mg)	530±271 (105-800) 307 (61-464)	423 [±] 217 (114-1190) 206 (56-583)
Pantothenic Acid (mg)	6.6±3.4 (3.6-11.8) 3.8 (2.1-6.8)	8.8±11.6 (2.4-69.0) 4.3 (1.2-33.8)
Alcohol (g)	0±0 (0-0) 0 (0-0)	<pre> 1[±]2 (0-12) 0 (0-6)</pre>

Table 9. Continued

a=mean[±]standard deviation of daily intake b=range of the daily intake c=nutrient intake per 1000 kcal d=range of the nutrient intake per 1000 kcal e=percent of RDA for the nutrient.

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According to the Physician's Desk Reference (Baker, 1981) only eleven of the twenty two listed prenatal multivitamins contained pantothenic acid and six of the eleven multivitamins had a level of 1.0 mg or less pantothenic acid per pill. The prenatal multivitamins contained pantothenic acid and those used by the subjects were Nataline Rx (Mead Johnson, 15 mg); Stuart prenatal with folic acid tablet (Stuart, 5.4 mg); Nunatal with fluoride (Mead Johnson, 1.0 mg); and Fosfree (Mission, 1.0 mg). Other dietary supplements used by the subjects which contained pantothenic acid were brewer's yeast and Nutrilites XX (Amway). The number of observations in which the subjects were taking pantothenic acid dietary supplements was only 11 out of the total 138 dietary observations. The number of subjects consuming the supplements were one, six and four at periods I, II and III, respectively.

The means and standard deviations of the pantothenic acid intake of the unsupplemented, the supplemented and the total population regardless of the treatment groups were 5.3 ± 1.8 mg (range 1.86-11.99), 42.9 ± 35.0 (range 10.3-108.5) and 8.3 ± 14.0 (range 1.8-108.5), respectively. Daily pantothenic acid intake from dietary supplements exclusive of diet ranged from 3.3 to 102 mg per day. A highly significant (p< 0.01) difference in pantothenic acid intake was observed between the supplemented and the unsupplemented populations regardless of the study group or the study period.

The number of individuals consuming pantothenic acid daily at levels less than 4 mg which is the lower limit of the Estimated Safe and Adequate Daily Dietary Intake for an adult, between 4 to 7 mg, and more than 7 mg a day are summarized in Table 10.

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lable.	10.	Number of	subjects consu	ming daily	pantotnenic acid
		at levels	of less than 4	mg, 4-7 mg	and more than 7 mg.

Intake		<4 mg		4	-7 mg		>7	mg	
Group	PI	PII	PIII	PI	PII	PIII	PI	PII	PIII
Nursing Bottle- Feeding	8	5	4 1	14 3	12 1	13 2	4	9 2	9
Control	8	4	4	7	10	10	2	3	3
Total	16	9	9	24	23	25	6	14	12
PI=Period	11.	PTT=Per	Tiod II.	PITT=Pe	eriod	TTT			

The proportion of individuals who consumed less than 4 mg pantothenic acid per day regardless of the group were 35%, 20% and 20% at period I, period II, and period III, respectively. The percentage of subjects consuming less than 4 mg daily in each group regardless of the period were 22%, and 31% of the nursing and the control group. Of those subjects, four individuals (three from pregnant and one from non-pregnant group) consumed less than 4 mg of pantothenic acid during the entire study period. The daily pantothenic acid intakes of the control, bottle feeding and nursing groups averaged 9.63, 6.04 and 7.71 mg, respectively (Table 11). The mean daily consumption of all groups at the period I, II and III were 7.21, 10.41 and 7.32 mg,

	a	II study subje	CTS.		
Group S	ubje (n)	ct Period I	Period II	Period III	Total
Control	17	10.47±1.92	9.91±1.92	8.49±1.92	9.63±1.11
Bottle- feeding	3	4.85±4.58	8.97±4.58	4.30±4.58	6.04±2.64
Nursing	26	6.54±1.58	10.90±1.56	6.89±1.56	8.11±0.90
Total	46	7.29±1.74	9.93±1.73	6.56±1.73	

Table 11. Observed means of total pantothenic acid intake: all study subjects.

Unit=mg/day Mean[±]standard deviation

Table	12.	Adjusted an	alysis	of var	iance	table	of
		pantothenic	acid	intake	: all	study	subjects.

Source	DF	Sum of Squares	Mean squares	F-ratio	Sig.Lev.
Period	2	130.9152	65.4576	1.04	ns
Period x Group	4	129.0264	32.2566	0.51	ns
Group	2	3.0318	1.5159	0.17	ns
Subject	43	20883.6298	485.6658	7.72	p<0.01
Error	86	5407.8861	62.8824		
Total	137	26554.4893			

ns=no significance

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Fig 2. Mean dietary intake vs period vs group: all study subjects.

respectively. No significant difference was observed among groups, periods and in interaction between group and period. A significant difference was noted (p<0.001) among the subjects. The pairwise comparisons resulted in no difference with the LSD test. The adjusted analysis of variance table of diet intake including all study subjects is shown in Table 12.

The means and standard deviations of pantothenic acid intake from food and dietary supplements of all study population are summarized in Table 11. The plot of mean pantothenic acid intake versus period versus group is shown in Fig 2.

Pantothenic Acid Intake of Subjects Consuming no Dietary Supplement

The observed mean intakes of pantothenic acid from the diet only of the control, the bottle-feeding and the nursing population regardless of the period were 4.80, 4.61 and 5.72 mg, respectively (Table 13). Although the overall group effect was insignificant in the analysis of variance (Table 14), pairwise comparisons among the three groups detected a significance between the nursing and the control group. The pregnant and nursing group consumed more pantothenic acid than the control or the bottle-feeding group did. The observed mean daily pantothenic acid consumption at the three periods regardless of the group were 4.96, 5.50 and 5.53 mg, respectively. No significant difference was observed among the three periods. No significant interaction between group and period was

Group	Period I	Period II	Period III	Total
Control	4.34±0.36	4.98±0.36	5.06±0.38	4.79±0.21
Bottle- feeding	4.85±0.83	4.54±1.65	4.30±0.83	4.56±0.68
Nursing	5.34±0.29	5.86±0.31	6.11±0.30	5.77±0.17
Total	4.84±0.32	5.12±0.57	5.15±0.32	

Table 13. Observed means of dietary pantothenic acid intake: group taking no supplement.

Unit=mg/day Mean±standard deviation

Table 14. Adjusted analysis of variance table of pantothenic acid intake: subjects taking no supplement.

DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
2	1.0789	0.5394	0.26	ns
4	2.4870	0.6218	0.30	ns
2	1.5936	0.7968	2.43	ns
42	232.7732	5.5422	2.70	p<0.01
76	156.1232	2.0543		
126	394.0559			
	DF 2 4 2 42 76 126	DF Sum of Squares 2 1.0789 4 2.4870 2 1.5936 42 232.7732 76 156.1232 126 394.0559	DF Sum of Squares Mean Squares 2 1.0789 0.5394 4 2.4870 0.6218 2 1.5936 0.7968 42 232.7732 5.5422 76 156.1232 2.0543 126 394.0559	DF Sum of Squares Mean Squares F-ratio 2 1.0789 0.5394 0.26 4 2.4870 0.6218 0.30 2 1.5936 0.7968 2.43 42 232.7732 5.5422 2.70 76 156.1232 2.0543 126 126 394.0559 394.0559 126

ns=no significance

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Fig 3. Mean dietary intake vs period vs group: subjects taking no supplement.

observed as shown in Table 14.

The means and standard deviations of the pantothenic acid intake from the diet only of the nursing, the bottle-feeding and the control population at three different periods are summarized in Table 13. A pairwise comparison among all combinations using the LSD test detected a significance in dietary intake between the nursing and control group at periods I and III.

The plot including the means of pantothenic acid intake versus period versus group is shown in Fig 3.

Blood and Plasma

The observed means and standard deviations of blood pantothenic acid levels of the subjects taking no dietary supplements, the subjects taking supplements and the total population regardless of period and group were 470.66±109.56 ng/ml (range 258.00-952,00 ng/ml), 730.28±154.55 ng/ml (range 514.71-1092.00 ng/ml) and 491.35±133.28 ng/ml (range 258.00-1092.00 ng/ml), respectively. The observed means and standard deviations of the plasma pantothenic acid levels of individuals consuming no dietary supplements, individuals consuming dietary supplements and the total subjects regardless of period or group were 108.81±22.42 ng/ml (ranged 59.17-181.67), 127.55±22.34 ng/ml (ranged 88.34-165.00) and 110.30±23.30 ng/ml (ranged 59.17-181.67) ng/ml, respectively.

Pearson's correlations between the blood and pantothenic acid intakes of subjects taking no supplement, subjects taking supplements and all population were significant at the level of 0.024 (n=127, r=0.1752), 0.043 (n=11, r=0.5399) and 0.000 (n=138, r=0.5169), respectively (Table 33, Table 34 and Table 35). The correlation between the dietary intake and blood level was significant at the level of p=0.05 in all of the groups. The test of Pearson's correlations between the plasma level and the pantothenic acid intake of subjects ingesting no supplements, subjects taking supplements and all study population were significant at the level of 0.214 (n=127, r=0.0710), 0.090(n=11, r=0.4370) and 0.006 (n=138, r=0.2442), respectively. A significant correlation between the plasma and pantothenic acid intake was observed only when all population were included (p<0.05) in the statistical analysis. The Pearson's correlations between blood and plasma pantothenic acid levels of the groups taking no dietary supplements, the group taking dietary supplements and the whole participants were r=0.0616 (n=127, p=0.246), r=0.1704 (n=11, p=0.308) and r=0.0878 (n=138, p=0.153), respectively. Statistically no correlation was found between the two variables. No significant difference was observed between the oral contraceptive users and non-users in their blood levels.

Subjects Taking no Supplement

The observed mean blood and plasma levels of the control, the bottle-feeding and the nursing groups who did not ingest dietary

supplements were 507.51, 111.02; 431.26, 98.42; 450.71, 108.38 ng per ml regardless of the period. As seen in the analysis of variance table for the blood (Table 15), a significant group difference was observed with no significant change with the periods. The blood levels were highly influenced by the dietary intakes (p<0.001) even though a large individual variation in the blood level was observed. When the mean blood levels of the three groups were pairwise compared, significant differences were observed between the control and the nursing groups at all three periods and between the control and bottle-feeding groups during the gravid period.

The fasting plasma levels however were not different among the groups and periods (Table 16). Besides, individual variation of the fasting plasma levels was not significant as that of the blood level. The pairwise comparisons of the plasma levels among the groups and the periods failed to reject the null hypothesis. No interaction was observed between the group and the period on pantothenic acid level in plasma.

The blood and plasma pantothenic acid values of different groups at different periods are summarized in Table 17 and Table 18. The means of pantothenic acid content in fasting blood and plasma were plotted against group and period in Fig 4 and Fig 5.

All Study Subjects

The observed mean blood and plasma pantothenic acid levels of all the control, the bottle-feeding and the nursing groups regardless of

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period were 530.14, 112.21; 496.17,101.80; 465.44,110.04 ng/m], respectively. As seen in the analysis of variance table for the blood pantothenic acid level (Table 19), the effect of overall period, the diet pantothenic acid intake on the blood level and individual difference were significant (p<0.05). The overall group effect and the interaction were suggestive (P=0.084, p=0.061, respectively). The pairwise comparisons among all period and group combinations detected a significantly lower blood pantothenic acid level of the nursing population than the control group during the gravid period. The lower blood level in pregnancy slightly increased at two weeks postpartum at a insignificant level but significantly increased at three months postpartum. The bottle-feeding group experienced a significantly increased blood pantothenic acid at two weeks postpartum (p<0.05), when the blood levels in the nursing population and the bottle-feeding group were significantly different (p<0.05). The fasting plasma levels were not statistically significant for any variables (Table 20). Pairwise comparisons among all the group and period combinations proved no difference in the values at all. Although an interaction between the period and the group was suggested in the blood level (p=0.06), no interaction was observed in plasma values.

The adjusted and observed mean values of pantothenic acid in fasting blood and plasma are summarized in Table 21 and Table 22. The means of pantothenic acid in fasting blood and plasma were plotted aginst group and period as in Fig 6 and Fig 7.

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Sources	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
Period	2	20378.17	10189.09	1.65	ns
Period x Group	4	31758.89	7939.72	1.28	ns
Group	2	3662.70	1831.35	0.30	p<0.05
Diet	1	144105.40	144105.40	23.28	p<0.01
Subject	42	785096.91	18692.78	3.02	p<0.01
Error	75	464278.71	6190.38		
Total	126	742780.78			

Table 15. Adjusted analysis of variance table of blood levels: subjects taking no supplement.

ns=no significance

Table 16. Adjusted analysis of variance table of plasma levels: subjects taking no supplement.

Source	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
Period	2	200.80	100.40	0.18	ns
Period x Group	4	952.55	238.14	0.42	ns
Group	2	348.98	174.49	0.31	ns
Diet	1	4.79	4.79	0.01	ns
Subjects	42	17118.31	407.58	0.73	ns
Error	75	42106.12	561.41		
Total	126	60731.55			

ns=no significance

Group	Period I	Period II	Period III	Total
Control	534.21±20.60	525.19±19.79	516.97±20.69	525.46±12.01
	504.63±187.40	514.79±91.23	502.83±105.52	507.51±132.60
Bottle-	402.87±45.52	428.55±90.98	507.31±45.87	446.24±37.39
feeding	388.67±31.39	423.33±**	476.50±45.62	431.26±54.44
Nursing	404.16±15.81	444.81±17.36	451.26±17.11	433.41±9.96
	408.42±82.03	462.68±90.38	485.05±82.34	450.71±89.90
Total	447.08±17.58 461.31±134.63	416.19 [±] 31.52 482.54 [±] 92.51	491.85±17.53 490.79±88.28	

Table 17. Estimated and observed means of fasting blood levels: subjects taking no supplement.

Mean[±]standard deviation

**: larger than a half of the mean. The bottom numbers denote observed means and standard deviations Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means(Xi.) were at total mean(X...).

Group	Period I	Period II	Period III	Total
Control	117.51±6.20	112.68±5.96	103.81±6.23	111.33±3.62
	117.34±14.26	112.62±27.27	102.59±11.10	111.03±19.61
Bottle-	96.65±13.71	89.02±27.40	103.54±13.81	96.40±11.26
Feeding	96.57±6.13	89.17±**	103.36±14.67	98.42±10.62
Nursing	108.93±4.76	112.91±5.23	102.46±5.15	108.10 [±] 3.00
	109.13±19.93	112.75±32.14	103.38±21.16	108.38 [±] 24.72
Total	107.70±5.29 111.21±18.10	104.87±9.49 112.11±29.71	103.27± 5.28 103.09±17.43	

Table 18. Estimated and observed means of fasting plasma levels: subjects taking no supplement.

Unit=ng/ml Mean±standard deviation

**: larger than a half of the mean. The bottom values denote the observed means and stadard deviations.

Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means(Xi.) were at total mean(X...).



Fig 4. Mean blood level vs period vs group: subjects taking no supplement.



Fig 5. Mean plasma level vs period vs group: subjects taking no supplement.

DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
2	71324.8183	33662.4091	4.19	p<0.05
〈 4	79978.9506	19994.7376	2.35	ns(p<0.06)
1	300343.7425	300343.7425	2.62	ns(p=0.08)
2	5457.7241	2728.8620	35.29	ns
43	804675.9266	18713.3936	2.20	p<0.01
85	723360.6801	8510.1256		
137	1985141.8422			
	DF 2 4 1 2 43 85 137	DF Sum of Squares 2 71324.8183 4 79978.9506 1 300343.7425 2 5457.7241 43 804675.9266 85 723360.6801 137 1985141.8422	DF Sum of Squares Mean Squares 2 71324.8183 33662.4091 4 79978.9506 19994.7376 1 300343.7425 300343.7425 2 5457.7241 2728.8620 43 804675.9266 18713.3936 85 723360.6801 8510.1256 137 1985141.8422	DF Sum of Squares Mean Squares F-ratio 2 71324.8183 33662.4091 4.19 4 79978.9506 19994.7376 2.35 1 300343.7425 300343.7425 2.62 2 5457.7241 2728.8620 35.29 43 804675.9266 18713.3936 2.20 85 723360.6801 8510.1256 137

Table 19. Adjusted analysis of variance table of blood levels: all study subjects.

ns=no significance

Table 20. Adjusted analysis of variance table of plasma levels: all study subjects.

Group	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
Period	2	421.1136	210.5568	0.38	ns
Period x Group	4	981.5043	245.3761	0.45	ns
Group	1	1531.4961	1531.4961	0.70	ns
Diet	1	424.3976	212.1987	2.79	ns
Subject	43	19388.7789	450.9018	0.82	ns
Error	85	46671.1452	549.0722		
Total	137	69418.4357			

ns=no significance

Gp	Period I	Period II	Period III	Total	
CT	523.08±22.54 539.18±230.69	507.88±22.46 519.81±90.73	530.08±22.38 531.44±127.53	520.35±19.31 530.14±157.90	1
BF	414.47±53.44 388.67±31.39	618.43 [±] 53.27 623.33 [±] 180.94	506.38±53.50 476.50±45.62	513.09±45.79 496.17±139.62	
NG	425.26±18.49 408.42±82.03	477.04±18.38 496.33±131,31	502.12±18.18 491.56±83.72	468.14±15.57 465.44±105.21	
	454.27±20.25 455.46±164.10	534.45±20.28 513.29±122.27	512.86±20.30 505.31±100.95		
	Unit=ng/ml CT=control gr BF=Bottle-fee NG=Nursing gr	oup ding group oup deviation			

Table 21. Estimated and observed means of pantothenic acid in blood: all study subjects.

Meanistandard deviation Bottom number denote observed vlaues.

Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means(Xi.) were at total mean(X...).

Grou	p Period I	Period II	Period III	Total
CT	117.74±5.72	113.74±5.71	103.04±5.67	111.51±3.00
	118.89±15.21	114.59±27.63	103.14±11.47	112.21±20.14
BF	98.41±13.57	105.15±13.53	105.50±13.59	103.02±7.11
	96.57±6.13	105.50±29.01	103.36±14.67	101.80±17.03
NG	109.93±4.70	114.54±4.67	105.83 [±] 4.62	110.10±2.42
	109.13±19.13	115.92±32.10	105.07 [±] 23.41	110.04±25.72
	108.69±5.14 111.92±18.52	111.14 [±] 5.15 114.75 [±] 29.80	104.79±5.16 104.25±19.02	
ann is a star offic	Unit=ng/ml CT=control gro BF=Bottle-feed	oup ling group		

Table 22. Estimated and observed means of pantothenic acid in plasma: all study subjects.

NG=Nursing group Mean[±]standard deviation Bottom number denote observed values. Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means(Xi.) were at total mean(X...).

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TIME

Fig 6. Mean blood level vs period vs group: all study subjects.



Fig 7. Mean plasma level vs period vs group: all study subjects.

Mi1k

A statistical analysis was performed with the pantothenic acid content of fore and hind milk samples collected from twenty-six nursing women at the two weeks postpartum and at three months postpartum.

The observed means of pantothenic acid values in pre- and post-milk of non-supplement (51 observation), supplement (7 observations) and total subjects (58 observations) were as in Table 23.

Pre-mlik Post-milk Group (microgram/ml) (microgram/ml) No supplements 2.478±1.049 2.331±0.886 (range 0.050-6.370) (range 0.160-4.840)Supplements 3.621±1.910 3.503±1.757 (range 1.000-5.660) (range 1.000-5.000) 2.473±1.078 Total 2.616±1.221 (range 0.050-6.370) (range 0.160-5.000)

Table 23. Mean pantothenic acid content of human milk.

Mean[±]standard deviation

Although the observed pantothenic acid values in the fore milk samples were consistently higher than those in the hind milk samples in all three groups, the difference between fore and hind milk samples was statistically insignificant. Dietary pantothenic acid

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supplementation (20-70 mg daily), however, significantly increased the pantothenic acid level in both the fore and hind milk samples (p<0.05). The pantothenic acid contents in both fore and hind milk were unchanged with progress of nursing from two weeks postpartum to four month postpartum. The individual difference was large, varying from 0.05 to 6.37 micro g/ml in pre milk and from 0.16 to 5.00 microgram/ml in post milk. Both pre- and post-milk had significant correlations (p<0.01) with dietary intake and plasma level (p<0.05). A significant correlation was observed between hind milk and blood level (p<0.05) but the pre milk value had a suggestive correlation with blood level (p=0.075).

Urine

The urinary pantothenic acid excretion was expressed in as 24 h excretion value and a ratio of pantothenic acid/ creatinine. The Pearson's correlation coefficient between the two expressions was 0.94 and was significantly correlated (p<0.001). The mean urinary pantothenic acid excretion levels of the subjects taking no supplements, subjects taking supplements and all study subjects regardless of the period and the group are summarized as in Table 24.

A highly significant difference in urinary excretion of pantothenic acid was observed between the subjects consuming no dietary supplements and the subjects taking supplements (p<0.001).

Subjects	0bs(n)	PA/Day	PA/Creatinine
No supplement	127	3.61±1.19 (range 1.47-6.96)	4.61 [±] 1.87 (range 1.82-10.00)
Supplement	11	12.12 [±] 5.88 (range 5.74-21.24)	15.47 [±] 8.02 (range 6.31-28.10)
Total subjects	138	4.07±2.58 (range 1.47-21.24)	5.20 [±] 3.50 (range 1.82-28.10)

Table 24. Urinary pantothenic acid excretion of the unsupplemented, the supplemented and the total study subjects.

Unit=mg/day Mean[±]standard deviation

The mean daily creatinine excretion of total population throughout all periods was 1.567 g. No difference was observed in creatinine excretion among the groups or among the periods. The urinary pantothenic acid excretion in both the expression was reflected by the dietary intake (p<0.01) at a highly significant level. As shown in Table 33, Table 34 and Table 35, fasting blood pantothenic acid level and milk pantothenic acid content had in general suggestive correlations with urinary pantothenic acid excretions

The analysis of variance tables of the daily pantothenic acid excretion and the ratio of pantothenic acid/ creatinine in groups of subjects taking dietary supplements and subjects taking no supplements are shown in Table 25, Table 26, Table 27 and Table 28. No effect of the group or the period was seen on the urinary pantothenic acid excretion.

Sources	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
Period	1	7.3031	7.3031	1.50	ns
Period x Group	2	1.9606	0.9803	0.20	ns
Group	2	1.3424	0.6712	0.14	ns
Diet	1	13.6110	13.6110	2.79	ns
Subject	42	46.4957	1.1070	0.23	ns
Error	78	380.9549	4.8840		
Total	126	451.6677			

Table 25. Adjusted analysis of variance table of daily pantothenic acid excretion: group taking no supplement.

ns=no significance

Table 26. Adjusted analysis of variance table of pantothenic acid excretion per creatinine: group taking no supplement.

Source	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
Period	1	8.7533	8.7533	0.97	ns
Period x Group	2	1.7435	0.8717	0.10	ns
Group	2	3.1640	1.5820	0.18	ns
Diet	1	10.9687	10.9687	1.22	ns
Subject	42	130.6546	3.1109	0.35	ns
Error	78	700.6467	8.9827		
Total	126	855.9308			

ns=no significance

Source	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev
Period	1	20.7735	20.7735	2,41	ns
Period x Group	2	1.9396	0.9698	0.11	ns
Group	2	1.2816	0.6408	0.46	ns
Diet	1	0.3025	0.3025	0.04	ns
Subject	43	133.5379	3.1055	0.36	ns
Error	88	757.7736	8.6111		
Total	137	915.6087			

Table 27. Adjusted analysis of variance table of daily pantothenic acid excretion: all study subjects.

ns=no significance

Table 28. Adjusted analysis of variance table of pantothenic acid excretion per creatinine: all study subjects.

Source	DF	Sum of Squares	Mean Squares	F-ratio	Sig.Lev.
Period	1	30.0741	30.0741	1.98	ns
Period x Group	2	6.3664	3.1832	0.21	ns
Group	2	5.2983	2.6491	0.28	ns
Diet	1	0.0022	0.0022	0.00	ns
Subject	43	268.2119	6.2375	0.41	ns
Error	88	1336.6746	15.1895		
Total	137	1646.6275			

ns=no significance

	Period I	Period II	Total	
Control	3.00±0.58 (2.72±0.84)	2.13±0.40 (2.08±2.30)	2.57±0.17 (2.30±1.94)	
Bottle- Feeding	3.43±1.28 (3.29±0.41)	3.18±1.15 (2.69±1.81)	3.30±0.41 (2.95±1.34)	
Nursing	3.26±0.44 (3.20±0.93)	2.02±0.35 (2.17±2.34)	2.64 [±] 0.14 (2.54 [±] 2.01)	
Total	3.23±0.49 (3.03±0.89)	2.45±0.42 (2.16±2.28)		

Table 29. Estimated and observed means of daily pantothenic acid excretion: group taking no supplement.

Unit=mg/day

Mean[±]standard deviation

Numbers in parenthesis denote the observed values.

Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means($X_{i..}$) were at total mean($X_{...}$).

Table 30. Estimated and observed means of pantothenic acid excretion per creatinine: group taking no supplement.

(1895) ************************************	Period I	Period II	Total	
Control	3.84±0.78 (3.59±1.93)	2.90±0.55 (2.88±3.40)	3.37±0.29 (3.13±2.96)	
Bottle- Feeding	4.33±1.73 (4.20±0.72)	3.98±1.56 (3.58±2.46)	4.15±0.69 (3.85±1.82)	
Nursing	3.93±0.60 (3.81±1.29)	2.65 [±] 0.48 (2.73 [±] 2.94)	3.29±0.23 (3.12±2.53)	
Total	4.03±0.67 (3.76±1.50)	3.17±0.57 (2.83±3.06)		

Unit=mg/day

Mean[±]standard deviation

Number in parenthesis denote the observed values.

Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means(Xi.) were at total mean(X...).

	Period I	Period II	Total
Control	3.82±0.72	2.49±0.50	3.15±0.26
	(3.81±4.57)	(2.48±3.08)	(2.92±3.65)
Bottle-	3.26±1.70	1.78±1.20	2.52±0.63
Feeding	(3.29±0.41)	(1.80±1.98)	(2.29±1.74)
Nursing	3.19±0.59	2.34±0.41	2.77±0.22
	(3.20±0.93)	(2.34±2.73)	(2.63±2.32)
Total	3.43±0.64 (3.43±2.83)	2.20±0.45 (2.35±2.81)	

Table 31. Estimated and observed means of daily pantothenic acid excretion: all study population.

Unit=mg/day

Mean[±]standard deviation

Number in parenthesis denote the observed values.

Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means($X_{i..}$).

Table 32. Estimated and observed means of pantothenic acid excretion per creatinine: all study population.

Children De Carlos de constantes	Period I	Period III	Total
Control	5.03±0.95	3.27±0.67	4.15±0.37
	(5.03±6.23)	(3.27±3.95)	(3.68±4.84)
Bottle-	4.20 [±] 2.26	2.39±1.59	3.29±0.89
Feeding	(4.20 [±] 0.72)	(2.39±2.65)	(2.99±2.31)
Nursing	3.84±0.78	3.00±0.54	3.42±0.30
	(3.81±1.29)	(3.00±3.62)	(3.27±3.06)
Total	4.36±0.86 (4.23±3.89)	2.89±0.60 (3.06±3.66)	

Unit=mg/day

Mean[±]standard deviation

Number in parenthesis denote the observed values.

Estimated means: Adjusted treatment means that are estimates of what the treatment means would be if all individual treatment means(Xi.) were at total mean(X...).



Fig 8. Mean daily pantothenic acid excretion vs period vs group: subjects taking no supplement.

Fig 9. Mean pantothenic acid per creatinine excretion vs period vs group: subjects taking no supplement.

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TIME

Fig 10. Mean daily pantothenic acid excretion vs period vs group: all study subjects.



Fig 11. Mean pantothenic acid per creatinine excretion vs period vs group: subjects taking no supplement.

The observed and estimated means and standard deviations of the urinary excretion of the group taking no dietary supplements and of the All Study Subjects at different periods are summarized in Table 29, Table 30, Table 31 and Table 32. The pairwise mean comparisons among each cells detected no statistical difference.

The plots among the means of daily urinary excretion and the pantothenic acid per creatinine ratio versus group versus period are shown in Fig 8, Fig 9, Fig 10 and Fig 11. The interpretations were the same between the group taking no supplements and the total study group and between the daily pantothenic acid excretion and the ratio of pantothenic acid per creatinine.

<u>Combined Data for Evaluation of</u> Pantothenic Acid Status

The correlation coefficients and significance level among the dietary pantothenic acid intake and other biochemical variables of thesubjects taking no supplement, the subjects taking supplements and the total study population are summarized in the Table 33, 34 and 35. A complete data file of each individual including all demographic and biochemical variables and dietary intakes are shown in Appendix F.

The three longitudinal treatment groups were re-divided into four cross-sectional physiological treatment groups: pregnant women in third trimester; lactating women postpartum between two weeks and three months; non-nursing postpartum women; and non-pregnant and non-nursing control group. The average pantothenic acid levels in the diet and in the biological fluids of all participants for the four groups are summarized in Table 36. Table 37 presents the same data of participants who did not take dietary supplements to eliminate the large deviation of subjects taking supplements. When data of the total study participants were compared, the twenty-nine pregnant women had significantly lower pantothenic acid levels in blood and urinary excretion eventhough their dietary intake was not statistically different from other groups, i.e., control women, nursing women and non-nursing women at postpartum. The twenty-six nursing women did not have significant difference in any variables from those of the control and the non-nursing postpartum women at two weeks postpartum and at three months postpartum.
Table 3	3. Pears acid group	on corre dietary taking	lation c intake a no suppl	oefficie nd bioch ement.	nts amon emical v	g the pan ariables:	tothenic
	Blood	Plasma	PA/Day	PA/CR	Fore-M	Hind-M	Diet
Plasma	-0.0616 (127) p=0.246						
PA/Day	0.2346 (87) p=0.014	-0.1042 (87) p=0.168					
PA/Cr	0.1698 (87) p=0.058	-0.0902 (87) p=0.203	0.7963 (87) p=0.000				
Fore-M	0.0161 (51) p=0.455	0.1664 (51) p=0.122	0.1661 (27) p=0.204	0.2700 (27) p=0.087	o		
Hind-M	0.0498 (51) p=0.364	0.1357 (51) p=0.171	0.2935 (27) p=0.069	0.4013 (27) p=0.019	0.8900 (51) p=0.000		
Diet	0.1752 - (127) p=0.024	-0.0710 (127) p=0.214	0.4802 (87) p=0.000	0.2948 (87) p=0.003	0.5163 (51) p=0.000	0.5034 (51) p=0.000	

Numbers in parenthesis denote the number of observations. p=significance level.

Table 34. Pearson correlation coefficients among the pantothenic acid dietary intake and biochemical variables: group taking supplements.

	Blood	Plasma	PA/Day	PA/CR	Fore-M	Hind-M	Diet
Plasma	0.1704 (11) p=0.308						
PA/Day	0.7803 (5) p=0.060	0.4647 (5) p=0.215					
PA/CR	0.7440 (5) p=0.075	0.3086 (5) p=0.264	0.9690 (5) p=0.003				
Fore-M	-0.0867 (7) p=0.427	0.1487 (7) p=0.375	1.0000 (2) p=***	1.0000 (2) p=***			
Hind-M	-0.1988 (7) p=0.335	0.2902 (7) p=0.264	-1.0000 (2) p=***	-1.0000 (2) p=***	0.9645 (7) p=0.000		
Diet	0.5399 (11) p=0.043	0.4370 (11) p=0.090	0.9730 (5) p=0.003	0.9070 (5) p=0.017	0.5723 (7) p=0.090	0.5932 (7) p=0.080	

Numbers in parenthesis denote the number of observations. p=significance level.

Table 35. Pearson correlation coefficients among the pantothenic acid dietary intake and biochemical variables: all study populations.

	Blood	Plasma	PA/Day	PA/CR	Fore-M	Hind-M	Diet
Plasma	0.0878 (138) p=0.153						
PA/Day	0.5587 (92) p=0.000	0.1624 (92) p=0.061					
PA/Cr	0.5171 (92) p=0.000	0.1409 (58) p=0.090	0.9416 (92) p=0.000	k			
Fore-M	0.1911 (58) p=0.075	0.2248 (58) p=0.045	0.5507 (29) p=0.001	0.6346 (29) p=0.000			
Hind-M	0.2199 (58) p=0.049	0.2391 (58) p=0.035	0.5931 (29) p=0.001	0.6416 (29) p=0.000	0.9203 (58) p=0.000		
Diet	0.5169 (138) p=0.000	0.2442 (138) p=0.002	0.6926 (92) p=0.000	0.8272 (92) p=0.000	0.4865 (58) p=0.000	0.5325 (58) p=0.000	

Numbers in parenthesis denote the number of observations. p=significance level.

Control	Pregnancy	Lactation	Non-nursing postpartum
Observation 51	29	52	6
Diet 9.63±19.74 (mg/day)	5.30±1.74	8.90±11.66	6.64±3.48
Blood 530.14±157.90 (ng/ml)	406.38±78.21ª	493.94±109.06	549.92±142.82
Plasma 112.21±20.14 (ng/ml) Urine:	107.83±19.30	110.50±28.35	104.43±20.59
(mg/day) 4.38±3.69 (mg/g cr)	3.21±0.88b	4.68±1.97	3.59±0.31
5.78 [±] 4.90 Milk	3.85±1.24ª	6.00±2.82	4.77±0.73
(microgram/ml)		2.80±1.15	
(microgram/ml)		2.64±1.01	

Table 36. Pantothenic acid levels in dietary intake and in the biological fluids: total study subjects.

Mean[±]standard deviation

a=The value is significantly lower than the control, lactation and the non-nursing group (p<0.05). b=The value is significantly lower than the control and the lactating group (p<0.05).

cr=creatinine

	Control	Pregnancy	Lactation	Non-nursing postpartum
Observati	on 47	29	46	4
Diet	4.80±1.64	5.30±1.73	5.92±2.01	4.43±0.54
Blood 50	07.51±132.60	406.38±78.21ª	474.10±86.17	463.21±45.77
Plasma 1 (ng/ml)	11.03 [±] 19.61	107.83±19.30	107.97±27.21	99.82±13.92
(mg/day)	3.49±1.24	3.21±0.88 ^b	4.25±1.32	3.59±0.31
Milk	4.74±2.35	3.85±1.24ª	5.36±1.63	4.77±0.73
(microgram Hind	m/ml)		2.60±1.00	
(microgram	m/ml)		2.44±0.83	

Table 37. Pantothenic acid levels in dietary intake and in the biological fluids: subjects taking no supplement.

Mean[±]standard deviation a=The value is significantly lower than the control, the lactation and the non-nursing group (p<0.05). b=The value is significantly lower than the control and the lactating group (p<0.05). cr=creatinine

CHAPTER V

DISCUSSION AND CONCLUSION

<u>General Nutritional Intake of</u> <u>Pregnant, Lactating and</u> <u>Control Women</u>

The seventeen non-pregnant and non-lactating women in the control group consumed daily means of 1802±807 kcal and 72.19±23.65 g proteins (Table 8). The bottle-feeding postpartum women consumed slightly lower intake of 1724±469 kcal and 59.78±15.78 g protein (Table 9) than the control women but the difference between the two groups was statistically insignificant. The energy and protein intakes of the groups in the present study were not statistically different from those of the United States Health and Nutrition Examination Survey (HANES), 1971-1974 (1979) in which the white women, ages 15 to 34, above poverty level of income (n=2589) consumed mean intakes of 1692 ± 719 kcal and 67 ± 24.1 g protein. The daily mean intakes from food and dietary supplement of other nutrients for which the RDA are established were above the standards except for the zinc intake. The women in the control group and in the bottle-feeding group consumed daily averages of 11.78±8.08 mg and 9.71±2.56 mg zinc which are only 78.5 % and 64.7 % of the RDA, respectively for the groups. The proportions of the daily total energy source were 16.0 %,

37.7 % and 46.8 % from protein, fat and carbohydrate, respectively.

The daily mean energy and protein intakes of the twenty-nine gravid women were 2073±467 kcal and 81.36±19.64 g, respectively (Table 8). The group consumed 100 % or above the RDA for protein, calcium, phosphorus, vitamin A, thiamin, riboflavin, niacin, pyridoxine, vitamin B_{12} and ascorbic acid. The average intake of magnesium, folic acid, iron and zinc were lower than the allowances for the group and were 364.43±119.64 mg (81.0 % RDA), 703.90±487.52 microgram (88.0 % RDA) and 33.13[±]28.84 mg (69.0 % RDA) and 13.03±5.89 mg (65.2 % RDA), respectively. The dietary intakes of the pregnant women in this study are comparable with findings of other studies. Williams et al. (1981) reported daily intakes of 2912 kcal, 77.6 g protein in twenty-five pregnant women. Picone et al. (1982) reported that eighteen pregnant women who were all nonsmokers and low socioeconomic status and had adequate weight gain consumed averages of 1905±322 kcal, 84±24 g protein. The macronutrient intakes of the other studies are not statistically different from the results of the present study. The micronutrient intakes of this group, however, were significantly higher than the levels of Picone et al. (1982) in which eighteen pregnant women consumed averages of 1.3±0.3 mg thiamin, 1.9[±]0.4 mg riboflavin, 114[±]76 mg ascorbic acid, 23[±]11 mg niacin, 13 ± 5 mg iron and 769 ± 270 mg calcium. The results of the present study are comparable with those of Roepke and Kirksey (1979) in which both welfare patients and private practice patients were studied and the total intakes from food and dietary supplements of vitamin A,

vitamin C, niacin, riboflavin, thiamin, vitamin B_6 and iron averaged 220 %, 300 %, 200 %, 200 %, 320 %, 250 % and 400 % of the RDA for the group, respectively. Roepke and Kirksey (1979) reported sub-standard intakes of magnesium (60 % RDA) and zinc (50 % RDA) in the pregnant women. The results agree with findings of this study. The intakes of magnesium and zinc of the gravid women in the present study averaged $364.43^{\pm}119.94$ mg (81.0 % RDA) and $13.03^{\pm}5.89$ mg (65.2 % RDA) and ranged from 172.92 to 688 mg and 2.12 to 13.78 mg, respectively.

The twenty-six nursing women consumed averages of 2016 kcal and 81 g protein (Table 9). The macronutrient intakes of this study agreed well with other studies (Blackburn and Calloway, 1976; Thomson et al. 1970; Sims, 1978). The intakes of protein, calcium, phosphorus, vitamin A, thiamin, riboflavin, niacin, pyridoxine, vitamin B_{12} , ascorbic acid and folic acid in this study were over the 100 % of the allowances for the group. Intakes of iron, magnesium and zinc, however, were less than the standard and met only 73.1 %, 60.8 % and 49.1 % of the RDA, respectively. These findings agree with those of Sims (1978) in which total intakes of most nutrients from food and supplements met the RDA for the group. In the study the intake of energy, protein, fat, carbohydrate, calcium, iron, vitamin A, thiamin, niacin and ascorbic acid were 2124±578 kcal, 94±23 g, 96±45 g, 228±63 g, 1568±478 mg, 61.2±35.4 mg, 13077±65 IU, 3.99±1.92 mg, 4.83[±]1.93 mg, 37.8[±]17.8 mg and 248[±]127 mg, respectively. The values were not statistically different from those of the present study. The value Sims (1978) reported was significantly higher than

the 28.66[±]25.50 mg iron from food intake plus supplements found in this study. The total intakes of vitamin B_6 and iron reported by West and Kirksey (1976) were 2.2 mg, 14.3 mg, respectively. and by Thomas and Kawamoto (1976) were 1.69 mg, 15.4 mg, respectively. These values were significantly lower than 3.35 mg, 28.66 mg of this study.

Pantothenic Acid Intake

The avarage daily total pantothenic acid intakes of the pregnant, the lactating, the bottle-feeding and the control women were 5.30±1.73 mg, 8.82±11.68 mg, 6.64±3.48 mg and 9.63±19.74 mg, respectivley (Table 36). Although the pantothenic acid intake of the control group was higher, statistically no significant difference was found among the four groups. The higher average values of the control women is suspected as due to an individual who consumend average daily supplements of 56.6 to 108.5 mg pantothenic acid throughout the study period. The average dietay pantothenic acid intake from the food only of the pregnant, the lactating, the bottle-feeding and the control women were 5.30±1.73 mg, 5.92±2.01 mg, 4.43±0.54 mg and 4.80±1.64 mg, respectively. The average dietary pantothenic acid intakes per 1000 kcal energy were 2.66 mg, 2.56 mg, 2.90 mg and 2.56 mg, respectively for the control, the pregnant, the lactating and the postpartum non-nursing women. No statistical difference was found among the mean daily intakes and among the pantothenic acid per 1000 kcal intake of the four groups.

The total pantothenic acid intake of 5.30[±]1.73 mg of the pregnant women is comparable with the result of Cohenour and Calloway (1972). They reported an average dietary pantothenic acid intake of 4.7[±]1.4 mg in seventeen pregnant women taking no dietary supplements and an average total pantothenic acid intake of 7.2±1.9 mg in thirteen pregnant women taking dietary supplements. Although the findings of this study were slightly higher than 4.7[±]1.4 mg intake of the unsupplemented pregnant group in the study of Cohenour and Calloway (1972), it was not statistically significant. The value was significantly lower than the 7.2[±]1.9 mg of supplemented group in the study of Cohenour and Calloway (1972). Since none of the women in this study took a supplement containing more than 1 mg of pantothenic acid during their pregnancy, the results from this study along with those of Cohenour and Calloway can reasonably well assess the pantothenic acid intake of a large pregnant population who are not taking food supplements. The mean dietary intake of the pregnant group was in the range of 4-7 mg pantothenic acid which is suggested for the normal healthy adult. Of the twenty-nine pregnant women, four subjects (14 %) ingested less than 4 mg/day, seventeen (59 %) took between 4-7 mg/day and only eight (28 %) consumed more than 7 mg/day. When the "higher" than the Estimated and Safe and Adequate Daily Dietry Intake of pantothenic acid suggested for pregnancy and lactation is interpreted as more than 7 mg/day, only 28 % of the subjects studied consumed the allowance. The average of pantothenic acid intake of 9,63±19.74 mg of the all women in the control group

was higher than the 6.7 mg intake of the eight women in the study of Fox and Linkswiler (1961).

The intake of 8.82[±]11.68 mg pantothenic acid of the nursing women of this study is comparable with the 8.03 mg pantothenic acid of ten lactating women in the study of Pratt et al. (1951). Although the average daily intake of the nursing women is slightly higher than that of the pregnant group, the variation became very large because of a subject taking dietary pantothenic acid supplements during the nursing period. Of the twenty-six nursing subjects, as a result, six subjects consumed less than 4 mg daily, thirteen ingested between 4-7 mg, and only six subjects consumed more than 7 mg. Only 26 % of the study group consumed above the Estimated Safe and Adequate Daily Dietay Intake for an adult.

The average daily pantothenic acid and energy intake of the nursing subjects taking dietary supplements and subjects taking no supplement were 42.88±34.99 mg, 2126.40±533.70 kcal and 5.32±1.84 mg, 1930.09±679.35 kcal, respectively. When the pantothenic acid values were expressed per 1000 kcal energy intake, it averaged 2.76 mg per 1000 kcal. These findings suggest that if a person choose foods similar to the subjects in this study, she would need to consume at least 1450 kcal to obtain a minimum of 4 mg pantothenic acid which is the lower limit of the Estimated Safe and Adequate Daily Dietary Intake for an adult. A pregnant women should consume at least 2550 kcal to obtain a minimum of 7 mg pantothenic acid daily. Overall a practice of wise food selection along with cptimum energy intake is suggested for the population. Particularly the suggestive (P=0.06) negative correlation between the intake of added sugar and pantothenic acid intake can be emphasized. Although statistically insignificant, both alcohol and carbohydrate intake correlated negatively with pantothenic acid intake.

Pantothenic Acid Levels in Biological Fluids

Correlations .

The pantothenic acid levels of blood , plasma and urine showed a large variations among individuals and among periods with the same subjects. The values were not correlated with any demographic variables such as age, height, weight or number of children or use of oral contraceptives. The correlation found between the blood pantothenic acid level and age in this study disagrees with the pantothenic acid study in women by Ishiguro (1972) who reported that the blood pantothenic acid level decreased with increasing age.

Although reports in the literature have emphasized the significant correlations between the dietary intake and urinary excretions (Fox and Linkswiler, 1961; Fry et al., 1976; Srinivasan et al., 1981), an attempt has seldom been made to correlate the dietary intake and the blood or plasma levels. Cohenour and Calloway (1972) determined the pantothenic acid level in the diet and blood but failed to correlate the two variables. Ishiguro (1962) reported an increase in the free pantothenic acid level in blood after supplementation of 60 mg pantothenic acid to pregnant women for a period of two months but found the bound pantothenic acid level of blood was not altered. Srinivasan et al. (1981) observed a moderate correlation between blood pantothenic acid level and dietary intake of the vitamin in non-institutionalized elderly subjects. In this study both blood and plasma levels correlated (p<0.05) with diet intake with r=0.52 and r=0.24, respectively, when all subjects were pooled (Table 32, Table 11). The urinary pantothenic acid excretions however correlated better with dietary intake. Both daily pantothenic acid excretion and pantothenic acid per creatinine were related to diet intake at p<0.01 with r=0.69 and r=0.83 respectively when all subjects were pooled. The correlation between diet intake and urinary excretion of this

study is close to the r=0.80 found in the study of Fox and Linkswiler (1961).

Pregnant Women

The pregnant women had a significantly lower blood pantothenic acid level with an average of 406.38±78.21 ng/ml (Table 31) than either the control group or the nursing group had. The findings agreed with those of Cohenour and Calloway (1972) and Ishiguro (1962). Cohenour and Calloway (1972) observed 1030±260 ng/ml of total pantothenic acid in blood of seventeen pregnant women and 1830±600 ng/ml in the blood of five non-pregnant women. The difference was significant. The authors' data suggested that the decrease in blood

total pantothenic acid of pregnant women was due to a decrease in the bound form pantothenic acid. They reported no difference in the free pantothenic acid levels between the two groups. Ishiguro (1962) reported a lower level of total pantothenic acid (655 ng/ml) in the blood of pregnant women than the control women (966 ng/ml) who were the same age as the pregnant women. He also found that the difference was mainly due to a decrease in the bound form of pantothenic acid without changes in the concentration of free pantothenic acid. When Ishiguro administered 60 mg of calcium pantothenate daily to the pregnant women, the free pantothenic acid content of the blood increased but the bound form of pantothenic acid was not changed even after two weeks of oral administration. Srinivasan and Belavady (1976) however reported different results. These authors observed 569.5±2.61 ng pantothenic acid per ml blood of pregnant women (n=17) and 626.4±55.27 ng/ml blood of the control group (n=8) and this difference was statistically insignificant.

In this study pregnant women had an average 107.83±14.30 ng pantothenic acid per ml of fasted plasma. The control group had a slightly higher level of 112.21±20.14 ng per ml, but the difference between the values of the two groups were statistically insignificant. The finding agree with that of Srinivasan and Belavady (1976). They reported 196.6±37.72 ng pantothenic acid per ml plasma of control group and 152.1±27.23 ng pantothenic acid per plasma of pregnant women but the difference was insignificant.

Although Cohenour and Calloway (1972), and Ishiguro (1962) did not report plasma or sera data, their reported free pantothenic acid values in the whole blood were very similar to the plasma values reported by Srinivasan and Belavady (1976) and this study. This is of particular interest because their reported mean total pantothenic acid values of whole blood varied from 569.5 ng/ml of Srinivasan and Belavady (1976) to 1120 ng/ml in the study of Cohenour and Calloway (1972) and no bound form of pantothenic acid was found in the plasma in this study. Cohenour and Calloway (1972) reported that pregnant women had an average of 80 ± 60 ng free pantothenic acid per ml of whole blood while the control women had an average of 60±60 ng free pantothenic acid per ml and the difference was insignificant. Ishiguro (1962) observed 100±36.5 ng of free pantothenic acid per ml whole blood for pregnant women and 118[±]5.12 ng of free pantothenic acid per ml whole blood in non-pregnant women and the difference was insignificant.

The pregnant women in this study excreted significantly lower levels of pantothenic acid than the control women even though their average daily dietary intake was not differnt from that of the control group (Table 36). The depressed urinary excretion was observed in both the total daily excretion $(3.85\pm0.88 \text{ mg/day})$ and the ratio of pantothenic acid/creatinine $(3.85\pm1.24 \text{ mg/g creatinine})$. This finding was contrary to that of Srinivasan and Belavady (1976). The authors found no significant difference in urinary excretion between the pregnant women $(2.0\pm0.25 \text{ mg/day})$ and a control group $(2.5\pm0.18$ mg/day). The disagreement between the results of the present study and those of Srinivasan and Belavady (1976) was speculated as due to a small sample size and the very large variation of the study of Srinivasan and Belavady (1976). The trends in the two studies were the same although the statistical significance was different. The results suggest that the average daily dietary pantothenic acid intake of 5.30[±]1.74 mg in this study was not sufficient to maintain normal level of the vitamin in the blood of pregnant women. Even when the blood level and urinary excretion of the pantothenic acid were depressed because of insufficient intake of pantothenic acid, the fasting plasma level was not changed. This result suggested the inadvisability of using plasma as an indicator of pantothenic acid

Nursing Women

The average blood pantothenic acid level during the nursing period (two weeks to three months postpartum) was significantly (p<0.05) increased (493.94 ± 109.06) from that of the third trimester of pregnancy (406.38 ± 78.21) and had no significant difference with that of the control women (Table 36). The twenty six nursing women had a lower blood pantothenic acid level $(496.94\pm109.06 \text{ ng/ml})$ than the three bottle-feeding postpartum women $(549.92\pm142.82 \text{ ng/ml})$, but the difference was statistically insignificant. Srinivasan and Belavady (1976) reported the same results in which the blood pantothenic acid level of the twelve nursing women (1-6 months)

postpartum) was 634.3±80.61 ng/ml while that of the eight control women was 626.4+-55.27 ng/ml. Cohenour and Calloway (1972) however reported differently. They observed the total blood pantothenic acid level of thirteen postpartum teenagers (six weeks postpartum, non-nursing) had a significantly depressed blood level (1120±260 ng/ml) than the control group (1830±600 ng/ml). The blood pantothenic acic level of the group was rather similar with that of the pregnant group. Perhaps, the mature grown women in this study have different physiological demands for pantothenic acid than the growing teenagers did. In addition the dietary intake of the present study was higher than that of the Cohenour and Calloway (1972). The average plasma pantothenic acid level of the nursing women was 110.50 ± 28.25 ng/ml which was a slightly higher value than that of the pregnant women (107.83±19.30 ng/ml) and lower than that of the control group (112.21[±]20.14 ng/ml) but was not statistically different among the groups. Srinivasan and Belavady (1976) observed the same effects in which the authors found no difference between the pantothenic acid level in eight nursing women, 1-6 months postpartum, $(193.1\pm24.50 \text{ ng/ml})$ and that of nine control women (196.6 ± 37.72) ng/ml plasma).

The urinary pantothenic acid excretion of the nursing women $(4.68\pm1.97 \text{ mg/day})$ was not statistically different from that of the control group $(4.38\pm3.69 \text{ mg/day}, 5.78\pm4.90 \text{ mg/g} \text{ creatinine})$ in both expressions of the daily total excretion and the pantothenic acid per creatinine ratio. Although no comparable control group data were

available, Pratt et al. (1951) reported an average of 4.4 mg daily pantothenic acid excretion with 8.03 mg intake in ten nursing women. The findings are very similar to those of the present study in which twenty six nursing women consumed 8.90 ± 11.66 mg and excreted 4.68 ± 1.37 mg daily.

The pantothenic acid contents in fore and hind mature milk were 2.80[±]1.15 microgram/ml and 2.64[±]1.01 microgram/ml respectively and were not statistically different between the fore and the hind milk samples. The levels were significantly correlated with maternal diet intake (p<0.05) with r=0.49 and r=0.53 respectively. The milk values of the vitamin were in the reported range as indicated in Table 2 (Srinivasan and Belavady, 1976; Dept. of Hth. and Social Security, 1977; Telegdy-kovats and Szorady, 1968; Karlin R., 1957; Pellegrini and Chiari, 1955; Pratt et al., 1951; Schmidt, 1950; Macy, 1949; Coryell et al., 1945; Williams et al., 1942). The average milk pantothenic acid values of this investigation however, are disparate from those of Johnston et al. (1981). The authors reported very high levels of an average 6.7 microgram (range 1.8-18.5) in fore milk and 6.7 microgram (range 1.9-19.5) in the hind milk. The correlations they observed between the maternal diet intake and the milk levels however are comparable with the results of this study. Johnston et al. (1981) reported a positive correlation (r=0.51) between the maternal pantothenic intake of the day preceding milk collection and the pantothenic acid content of the milk.

No study has ever reported a correlation between the maternal circulating level of the vitamin and that of human milk. In the present study, the ratio of pantothenic acid content in human fore and hind milk samples over the vitamin level in the whole blood were 5.67 (2.8 microgram in fore milk per 493.84 ng/ml in whole blood) and 5.35 (2.64 microgram in hind milk per 493.94 mg in whole blood). The ratios were even larger when the milk values were compared with that of fasting plasma. The fore milk contained 25.34 times higher pantothenic acid concentration per volume and the hind milk contained 23.89 times higher values than the plasma level. This effect of higher vitamin concentration in human milk than in maternal circulation has been observed with other water-soluble vitamins, however, the ratio were larger with pantothenic acid than for other water-soluble vitamins. The correlations between the pantothenic acid levels of the fore and the hind milk and the vitamin level of the plasma were significant (p<0.05) when all samples were pooled regardless of the dietary supplement use for the purpose of statistical analysis (Table 11). The blood level was significantly (p<0.05) correlated with the vitamin content of the hind milk but suggestively (p=0.075) correlated with the content of the fore milk. When we assume the nursing women produce an average 850 ml of milk during the first three months postpartum, this will be equivalent to 2.31 mg pantothenic acid per day. This pantothenic acid consumption by infants is higher than the Estimated Safe and Adequate Daily Dietay Intake of 2 mg per day for the group, ages between 0-6 months.

Control Women

The average pantothenic acid levels of blood, plasma and urinary excretions of the control group in this study were 530.14±157.90 ng/ml, 112.21±20.14 ng/ml, 4.38±3.69 mg/day and 5.78 mg/g creatinine respectively. The values were not changed significantly during the three study periods of approximately three months interval. Fox and Linkswiler (1961), reported an intake of 6.7 mg pantothenic acid and excretion of 3.9 mg pantothenic acid in eight women, aged 18-24. The mean excretion of this study was slightly higher than that of Fox and Linkswiler (1961), so was the dietary intake. Cohenour and Calloway (1972) observed an average pantothenic acid excretion of 2.5 mg when five non-pregnant women consumed an average of 3.3 mg pantothenic acid. The intakes and urinary excretion levels of Cohenour and Calloway (1972) were significantly lower than those of the present study. The blood level of the five non-pregnant women in the study of Cohenour and Calloway (1972) was 1830 ng/ml. Since the pantothenic acid values of Cohenour and Calloway (1972) study were uncomparably high, the values were not directly compared with those in this study.

Conclusion

The average of total daily pantothenic acid intake of 5.30[±]1.74 mg for pregnancy observed in this study was lower than the nutrient allowance for the group. Their blood level and urinary excretion were

 $(406.38\pm78.21 \text{ ng/ml}, 3.21\pm0.88 \text{ mg/day})$ significantly lower than the control group. The average of total daily pantothenic acid intake of 8.90 ± 11.60 mg during lactation observed in this study was evaluated as appropriate for the group. The pantothenic acid levels in the blood and urinary excretion of the group $(493.94\pm1.97 \text{ ng/ml}, 600.00\pm2.82 \text{ mg/day})$ were not significantly different from those of the control group. Since no statistical difference was observed between the pantothenic acid intakes during pregancy and lactation pantothenic acid requirement during the third trimester of pregnancy appears to be higher than during the nursing period (2 weeks to 3 months postpartum). Postpartum bottle-feeding women maintained their blood level and urinary excretion which are not different from those of the control group with the daily pantothenic acid intake of $6.64\pm3.48 \text{ mg}$.

The dietary pantothenic acid intake of the population studied averaged 2.75 mg per 1000 kcal and no significant difference was found among the groups.

The levels of blood and urinary excretion were significantly correlated to the dietary intakes whereas the level of plasma was not. Thus, plasma pantothenic acid level was suggested as a poor nutritional indicator of the pantothenic acid intake or pantothenic acid status of an individual.

Human milk pantothenic acid levels were significantly reflected by the maternal dietary intake (r=0.63, p<0.001). The mean pantothenic acid levels in the fore milk and in the hind milk were 2.80 and 2.64 microgram/ml, respectivley and no significant difference was observed between the two types of milk samples.

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APPENDICES

Appendix A. Informed Concent Form

Pantothenic Acid Nutritional Status of Human

Principal Investigator - Won O. Song

- 1. The project includes analysis of blood, urine, breast milk and dietary intake of the subjects. A fasting blood drawn by venipuncture, urine samples, breast milk collected at different stages of lactation and dietary records for a week will be analyzed for pantothenic acid, its metablites and/or other components.
- 2. A venipuncture of subjects may result in discomfort, hematoma and a possibility of infection. Experience, however, has been that hematoma and discomfort can be significantly reduced when a trained medical technologist is involved. Sterile and standard procedure will be followed.
- 3. An individual will obtain an assessment of the nutritional adequacy for her normal diet and, if necessary, dietary counseling. Since hemoglobin and hematocrit will also be determined, any subject with abnormal values will be referred to her physician. The results obtained in the present project would enable the establishment of a requirement of pantothenic acid in the group.
- 4. It might be noted that the procedures to be employed in the present project appear to be least complicated and it would be impossible to draw conclusions by other procedures.
- 5. Subjects have the opportunity to withdraw consent and terminate participation in the activity at any time.
- 6. Emergency medical services would be available during the normal school hours at the Student Health Service, University Center, Utah State University.
- 7. Any inquiries concerning the project procedures would be answered anytime by the principal investigator or by other staff members associated with the project.

Subject, Signature and date PI, Signature & date

Auditor signature & date (if appropriate)

Appendix B. Research Protocol

STATEMENT OF PI TO THE IRB FOR PROPOSED RUSEARCH INVOLVING HUMAN SUBJECTS

Proposal Title Pantothenic acid status in lactating women and its content in
their breast milk
Primary Researcher Drs. B. W. Wyse & R. G. Hansen Dept. Nutrition & Food SciExt. 2123
Student Researcher* Won O. Song Dept. Nutrition & Food SciExt. 2117
A. Fullowing are the potential benefits to be gained from the proposed research:
This study will give data on which the recommended dietary allowances (RDA) of pantothenic
acid for the nutritionally high risk group can be based. The group's other nutritional
intake besides pantothenic acid will also be evaluated.
6. The risk(s) to the rights and welfare of human subjects involved are:
A venipuncture of subject may result in discomfort and a possibility of infection.
Collection of urine and breast milk and dietary record should not pose any risk to the
 <u>subjects.</u> The questionnaire form which is attached includes only non-invasive information. Therefore no real risk is foreseen. C. The following safeguards/measures to mitigate/minimize the identified risks will be taken: [] A fasting blood will be drawn by venipunture by a registered medical
technologist using sterile and standard procedures. 2) The individual's demographic
information will be delt with confidentially.
D. The lafe and forward and for a birth will be a fully of failed and

D. The Informed Consent procedures for subjects will be as follows: (Explain procedures to be used and attach an example of the instrument):Candidates for the study subjects will

be initially contacted by telephone at which time they will be informed of the study (purpose,

involvement, responsibility and benefits of the study) and will be asked for voluntary cooperation. A written consent form will be signed by each participant at first interview. L. The following measures regarding confidentiality of subjects will be taken: ______

All questonnaires will be collected and kept by the principal investigator. After first

interview, a numbering system will replace the subject's name.

F. Other (If in your opinion no, or minimal, risk to subjects exists, please explain in this section): <u>Collections of 8-10 ml of breast milk by using hand or breast-pump</u>.

Collections of urine pool or random urine samples for a week. Keeping dietary records

for a week FI Signature rite h

blucent Researcher signature

Appendix C. Questionnaire

Name	Subject Code
Address	Date .
Phone	
Age	
Physician	
Expecting Date	
Height:	
Weight (before pregnancy): lbs.	
(present):@wks.	
How do you want to feed your infant? Breast	Bottle Both
How many children do you have?	
Vitamin or mineral supplements: Yes Brand n	name
Did you take contraceptive pill before this pregname	ncy? YesNo
Other medications? Yes No	
If yes, what kind and brand:	
POSTPARTUM DATA:	
Feeding method: Breast Bottle	Both
Months intend to breastfeed	
Method of milk collection:	
Hand expression Breast pump	
Are you following weight reduction diet: Yes_	No
If yes, what calorie level	
Current weight	
Appendix D. Diet Record Form

FOOD RECORD

Name:

Date:

Instructions:

- 1. List the foods you eat for two consecutive days.
- Record EVERYTHING you eat or drink in each 24-hour period. Remember to write down such items as coffee, tea, cream, sugar, juice, milk, butter, margarine, jelly, gravy, mayonnaise, ketsup, mustard, pickles, soft drinks, alcohol etc.
- 3. Describe how the food was prepared and eaten (e.g., boiled, fried, or baked) and give a brand name when possible.
- Record the amount of each food and beverage in terms of units such as: cup(s), ounce(s), Tablespoon(s), teaspoon(s) or slice(s).

EXAMPLE

BREAKFAST	Amount
Whole wheat toast Margarine Apricot jam Egg, fried	l slice l tsp l tsp l
Orange juice, unsweetened (6 oz) Milk 2% (8 oz)	1 cup
MORNING SNACK	i cup
Postum Cream Sugar	l cup l tsp l tsp
LUNCH	
Chicken rice soup Sandwich made with: White bread Tuna Lettuce Mayonaaise Root beer, diet (12 oz)	l bowl 2 slices ½ cup one leaf 1 tsp 1 can
SUPPER	

complete as above

							Dates	
			D	IET RECORD FORM				
MEAL	FOOD	AMOUNT	MEAL	FOOD	AMOUNT	MEAL	FOOD	AMOU
<u>Breakfast</u>			Lunch			<u>Supper</u>		
Morning Snack			<u>Afternoon S</u>	nack				
						Evening Snack		

among All Nutrients

1		R Y B	U N O S	URRELA	TIDN	COEFFI	CIENT	1 1	1 1 1	
	NUTI	NUTZ	NUT3	NUT 4	NUTS	NUTS	NUT7	NUTB	NUT9	NUTIO
1 ION	(138)	(130)	(128)	0. 90 44	0.4414	(138)	0.3945	0.6120	0.0169	0.05050
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0. 605	P=0.000	P=0.000	P=0. 422	P=0.273
NUT2	0.7324	1.0000	0.7196	0.6616	C. 6352	0.2998)	0.5907	0.8195	0.2095	0.1936
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.000	P=0.000	P=0.030	P=0.000	P+0.000	P=0.000	P=0.000	P=0.007	P=0.011
NUT3	0.7733	0.719n	1.0000	0.5704	0.4720	0.1461	C. 3612	0.5820	0.0524	0.1351
	(138)	(158)	1351	(136)	(138)	(138)	(138)	(136)	(138)	(138)
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P.0.044	P=0.000	P=0.000	P=0.271	P=0.037
NUT 4	0.9044	0.6016	0.5704	1.0000	0.3272	0.1800	0.3063	0.4173	-0.0634	-0.0541
	(138)	(138)	(:351	(138)	(136)	(133)	(136)	(138)	(138)	(138)
	P=0.000	F=0.000	P=0.000	P=0.000	P=0.003	F.0.017	P=0.000	P=0.000	P=0.230	P=0.264
NUTS	0.4914	0.6352	0.4720	0.3272	1.0000	0.3606	0.7450	0.8500	0.4401	0.5128
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
NUT6	0.2152	0.2948	0.1461	0.1600	0.3606	1.0000	0.5473	0.3373	0.5480	0.3978
	(135)	(138)	1351	(133)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.006	P=0.000	P=0.044	P=0.017	P=0.000	P*0.000	P=0.000	P=0.000	P=0.000	P=0.000
NUT7	0.3445	0.5907	0.3612	0, 3083	0.7450	0.5473	1.0000	0.8132	0.5448	0. 6351
	(138)	(138)	(136:	(138)	(138)	(138)	(138)	(133)	(138)	(133)
	P=0.000	P=0.000	P=0.000	P=0, 000	P=0.003	PLO.000	P=0.000	?=0.000	P=0.000	P=0.000
NUTB	0.6120	0.8195	0.5920	0.4173	0.8500	0.3373	0.6132	1.0000	0.3962	0. 3057
	(138)	(135)	(138)	(138)	(138)	(136)	(133)	(138)	(138)	(138)
	P=0.000	P=0.003	P=0,000	P=0.000	P=0.000	P-6.000	P=0.000	P=0.000	P=0.000	P=0.000
NUT9	0.0169	0. 2095	0.0524	-0.0634	0.4401	0.5480	0.5448	0.3962	1.0000	0.5866
	(138)	(133)	(139)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.422	P=0. 007	P=0.271	P=0.230	P=0.000	Pro.000	P=0.000	P=0.000	P=0.000	P=0.000
NUT10	0.0505	0.1936	0.1351	-0.0541	0.5128	0.3978	0.5351	0.3039	0.3856	1.0000
	(138)	(138)	(138)	(138)	(138)	(133)	(138)	(138)	(138)	(138)
	P=0.278	P=0.011	P=0.057	F=0.264	P=0.000	Pr0.000	F=0.000	P=0.000	P=0.000	P=0.000
NUT11	0.0916	0. 2318	0.1549	-0.0060	0.5621	0.4416	0.6720	0.5374	0.6227	0.9372
	(138)	(138)	(:351	(138)	(136)	(138)	(138)	(138)	(138)	(138)
	P=0.143	P=0.003	P=0.025	P=0.472	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
NUT12	0.1062	0. 2600	0.1507	0.0010	0.4965	0.7372	0.5525	0.4846	0.6647	0.8314
	(138)	1 138)	1351	(153)	(136)	(139)	(138)	(138)	(138)	(138)
	P=0.108	P=0. 001	P=0.030	P=0.495	P=0.000	P+0.000	P=0.000	P=0.000	P=0.000	P=0.000
C I TUM	0.0492	0.2024	0.1219	-0.0530	0.5174	0.5658	0. 6401	0.4837	0.6448	0.9410
	(138)	(138)	1371	(138)	(136)	(138)	(138)	(138)	(138)	(138)
	P=0.283	P=0.004	P=0.07	F=0.268	P=0.000	P. 0.000	P=0. 000	?=0.000	P=0.000	P=0.000
NUT14	0.1036 (851) P=0.113	0.2966 (13H) P=0.000	650°0=4	0.0234 (138) F=0.392	000.0 -9 .	0.7171 (138) P+0.000	0.4526 (138) P=0.000	0.374B (138) P=0.000	0.7106 (138) P=0.000	0.4565 (138) P=0.000

NUT15	0.0791	0.2036	0.0675	0.0290	0.5330	0,4032	0.6881	0. 5106	0.5538	0.7986
	P=0.178	P=0.008	P=0.216	P=0.368	P=0.000	P=0.000	(138) R=0.000	(138)	(138)	(138)
NUTIA	. 0 2091	0.0444					1-0.000	1-=0.000	P=0.000	P=0.000
	(1201	0.3544	0.2215	0.1134	0.4823	0.8628	0.6368	0 5084	0 (17)	
	R=0 007	(138)	(138)	(138)	(138)	(138)	(138)	(120)	0.01/6	0.6285
	F=0.007	P=0.000	P=0.005	P=0.093	P=0.000	P=0.000	P=0.000	P=0.000	(138) P=0,000	(138) R≡0 000
NUT17	0.4167	0.1416	0 2455	0 4900	0.0774					1-0.000
	(138)	(138)	(138)	0.4700	0.0776	0.0768	-0.0760	0.0543	-0.1812	-0.1215
	P=0.000	P=0.049	P=0.001	R=0.000	(138)	(138)	(138)	(138)	(138)	(138)
				1-0.000	P=0.183	P=0.185	P=0.188	P=0.264	P=0.017	P=0.073
NUT18	0.7438	0.6713	0.9565	0.5523	0 3985	0.13/3				
	(138)	(139)	(132)	(138)	(138)	0.1363	0.3066	0.5037	0.0372	0.1392
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.055	(138) P=0.000	(138)	(138)	(138)
NUT19	0, 5750	0 4052	0 /766					1-0.000	F=0.332	P=0.052
	(138)	(138)	0.6700	0.5061	0.1516	0.1027	0.2033	0.2294	-0.0543	-0 0174
	P=0.000	P=0.000	R=0.000	(138)	(138)	(138)	(138)	(138)	(138)	(120)
		0.000	F=0.000	P=0.000	P=0.038	P=0.115	P=0.008	P=0.003	P=0.264	P=0 420
NUT20	0.6306	0.6402	0.8643	0 4774	0 5000					1-0.420
	(138)	(138)	(138)	(122)	0. 5000	0.1075	0.2786	0.5549	0.0626	0.1301
	P=0.000	P=0.000	P=0.000	R=0.000	(138)	(138)	(138)	(138)	(138)	(138)
			1 -0. 500	F=0.000	P=0.000	P=0.105	P=0.000	P=0.000	P=0.233	P=0.064
NUT21	0.3744	0.4646	0.2657	0.2760	0 7294	0.0000				
	(138)	(138)	(138)	(138)	(130)	0.2899	0.8007	0.7459	0.4349	0.5535
	P=0.000	P=0.000	P=0.001	P=0.001	R=0.000	(138)	(138)	(138)	(138)	(138)
					1-0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000
NUT22	0.2280	0.3062	0.1502	0.1930	0.3493	0 9940				
	(138)	(138)	(138)	(138)	(139)	0. 7788	0.54/4	0.3480	0.5383	0.4020
	P=0.004	P=0.000	P=0.030	P=0.012	P=0.000	R: 0 000	(138)	(138)	(138)	(138)
					1 -0.000	F-0.000	P=0.000	P=0.000	P=0.000	P=0.000
N0123	0.6232	0.5708	0.4327	Ũ. 5578	0.5290	0 1991	0 4444			
	(138)	(138)	(138)	(138)	(138)	(130)	0.4115	0.4830	0.0850	0.0325
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0 013	(138) R=0.000	(138)	(138)	(138)
NUTDA	0.1701					1-0.015	F=0.000	P=0.000	P=0.161	P=0.353
NOT24	0.1784	0.4148	0.2375	0.0506	0.5295	0.5493	0 6150	0 5500		
	(138)	(138)	(138)	(138)	(138)	(138)	(120)	0. 5593	0.5723	0.7594
	P=0.018	P=0.000	P=0.004	P=0.278	P=0.000	P= 0.000	P=0.000	8=0.000	(138)	(138)
NUT25	0.2395	0 4404						-0.000	F=0.000	P=0.000
	(138)	0.4004	0.4093	0.0661	0.4295	0.2423	0.3562	0.4750	0 2414	
	P=0.002	R=0.000	(133)	(138)	(138)	(138)	(138)	(138)	(139)	0.4587
		-0.000	P=0.000	P=0.220	P=0.000	F=0.002	P=0.000	P=0.000	P=0.000	(138) P=0 000
NUT26	0.0877	0.2934	0. 2009	-0.05/0			3			0.000
	(138)	(138)	(138)	-0.0349	0.4700	0.2700	0.6015	0.5693	0.4771	0.8765
	P=0.153	P=0.000	P=0.005	P=0 254	(138)	(138)	(138)	(138)	(138)	(138)
				0.234	F=0.000	P=0.001	P=0.000	P=0.000	P=0.000	P=0.000
NUT27	0.0077	-0.0455	-0.1604	0.0058	-0.0700	-0.0448	0.000/			
	(138)	(138)	(139)	(138)	(138)	-0.0649	-0.0231	-0.0515	-0.0258	-0.0691
	P=0.464	P=0.298	P=0.030	P=0.473	P=0 207	P=0 225	(138)	(138)	(138)	(138)
			1		0. 20,	F. C. 223	P=0.394	P=0.274	P=0.382	P=0.210
COFFETC	IENT / CADES	1 01-01-01-01-01-01-01-01-01-01-01-01-01-0								
. COLFFIC	CASES)	/ SIGNIFIC	SANCE)	(A VALUE OF	F 79.0000 1	S PRINTED I	A COEFFIC	IENT CANNOT	RE COMPLETE	
									DE CUMPUTE	

	NUT11	NUT12	NUT13	NUT14	NUT15	NUT16	NUT17	NUT18	NUT19	NUT20
NUT1	0.0916	0.1062	0.0492	0.1036	0.0791	0.2091	0.4167	0.7438	0 5750	0 4304
	(139)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.143	P=0.103	P=0.283	P=0.113	P=0.178	P=0.007	P=0.000	P=0.000	P=0.000	P=0.000
NUT2	0.2318	0.2600	0.2024	0.2966	0.2036	0 3644	0 1414	0 1710		
	(138)	(138)	(130)	(138)	(138)	(120)	0.1416	0.6/13	0.4053	0.6402
	P=0.003	P=0.001	P=0.009	P=0.000	P=0.008	P= 0.000	P=0.049	(138)	(138)	(138)
							1-01047	1-0.000	F=0.000	P=0.000
NUT3	0.1549	0.1603	0.1219	0.1132	0.0675	0.2215	0.2625	0.9585	0.4755	0 8443
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(120)
	P=0.035	P=0.030	P=0.077	P=0.093	P=0.216	P=0.005	P=0.001	P=0.000	P=0.000	P=0.000
NUTA	-0.0060	0.0010	-0.0520	0 0004	0.0000					
	(138)	(139)	-0.0000	0.0234	0.0290	0.1134	0.4900	0.5523	0.5061	0.4236
	P=0.472	P=0 405	R=0 2(4)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	1-0.472	F=0.475	P=0.268	F=0.392	P=0.368	P: 0.093	P=0.000	P=0.000	P=0.000	P=0.000
NUT5	0.5621	0.4965	0.5174	0.3390	0. 5330	0.4823	0 0774	0.0005	0.151	
	(138)	(138)	(138)	(138)	(138)	(138)	(120)	0.3785	0.1516	0.5000
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	Pt 0 000	R=0 100	1387	(138)	(138)
						1-0.000	F=0.163	P=0.000	P=0.038	P=0.000
NUT6	0.4416	0.7372	0.5858	0.7171	0.4032	0.8628	0 0768	0 1343	0 1007	0
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(1333	0.102/	0.1075
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0, 185	P=0.056	P=0 115	(138)
	0 1750	1000							-0.115	1-0.105
NUT	0.6720	0.6525	0.6401	0.4526	0.6881	0.6368	-0.0760	0.3066	0.2033	0.2786
	(138)	(138)	(138)	(138)	(136)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0.000	P=0.188	P=0.000	P=0.008	P=0.000
NUTB	0. 5374	0.4848	0 4920	0 2740	0. 510/		-			
	(138)	(138)	(120)	0.3/48	0.5108	0.5095	0.0543	0.5037	0.2294	0.5547
	P=0 000	R=0.000	R=0.000	(138)	(138)	(138)	(138)	(138)	(138)	(138)
		-0.000	F=0.000	F=0.000	P=0.000	P=0.000	P=0.264	P=0.000	P=0.003	P=0.000
NUT9	0.6227	0.6647	0.6448	0.7106	0 5520	0 4174	0 1010			
	(138)	(138)	(138)	(138)	(138)	(130)	-0.1812	0.0372	-0.0543	0.0626
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	(138) P=0 017	(138)	(138)	(138)
							-0.017	F=0.332	P=0.264	P=0.233
NUT10	0.9872	0.8314	0.9410	0.4566	0.7986	0.6285	-0.1215	0, 1392	-0.0174	0 1201
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(170)	(1301
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0.000	P=0,078	P=0.052	P=0.420	P=0.064
NUT11	1.0000	0 8338	0.0510	6 E440						
	(138)	(120)	0.4514	0.5140	0.8052	0.6260	-0.1075	0.1556	-0.0152	0.1667
	R=0.000	(138) R=0.000	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	1-0.000	F=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P=0.105	P=0.034	P=0.430	P=0.025
NUT12	0.8339	1.0000	0.8635	0.7025	0.6752	6 8825	-0.0447	0.4440		
	(138)	(138)	(138)	(138)	(178)	(130)	-0.0467	0.1613	0.0524	0.1331
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	R=0.000	(138)	(138)	(138)	(138)
						1-0.000	F=0.273	P=0.029	P≈0,271	P=0.060
NUT13	0.9519	0.8636	1.0000	0.5856	0.7687	0.7284	-0.0681	0 1184	-0 0247	0 10/7
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(130)	(120)	0.136/
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	F= 0.000	P=0.214	P=0.083	R=0 37P	R=0.055
								-0.083	0.378	F=0.033
NUT14	0.5140	0.7025	0.5956	1.0000	0.4064	0.7103	-0.0291	0.0849	0.0434	0, 1283
	(138)	(130)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0.000	P=0.367	P=0.161	P=0.287	P=0.067

NUT15	0.8052 (138) P=0.000	0.6752 (138) P=0.000	0.768" (138) P=0.000	0.4064 (138) P=0.000	1.0000 (138) P=0.000	0.5964 (138) P=0.000	-0.0295 (138) P=0.365	0.0453 (138) P=0.299	-0.0160 (138) P≖0.426	0.0625 (138) P=0.233	
NUT16	0.6260	0.8935 (138)	0.7204 (138)	0.7103 (138)	0.5964 (138)	1.0000 (138)	0.0054 (138)	0.1987	0.1139	0.1674	
	P=0.000	P=0.000	P=0,000	P=0.000.	P=0.000	P=0.000	P=0.475	P=0.010	P=0.092	P=0.025	
45											
					*						
NUT17	-0.1075	-0.0467	-0.06E1	-0.0291	-0.0295	0.0054	1.0000	0.2402	0.1533	0.2563	
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	
	P=0.105	P=0.293	P=0.214	P=0.367	P=0.365	P= 0. 475	F=0.000	P=0.002	P=0.036	P=0.001	
NUT18	0.1556	0.1613	0.1186	0.0849	0.0453	0.1987	0.2402	1,0000	0. 5425	0 8217	
	(138)	(138)	(135)	(138)	(138)	(138)	(138)	(138)	(136)	(138)	
	P=0.034	P=0.029	P=0.083	P=0.161	P=0.299	P=0.010	P=0.002	P=0.000	P=0.000	P=0.000	
NUT19	-0.0152	0.0524	-0.0267	0.0484	~0.0160	0 1139	0 1823	0 4 4 7 4			
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	1.0000	0.3167	
	P=0.430	P=0.271	P=0.378	P=0.287	P=0.426	P= 0.092	P=0.036	P=0.000	P=0.000	P=C. 000	
NUT20	0.1667	0. 1331	0.1357	0.1283	0.0625	0.1674	0.2543	0 8217	0 3147		
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(128)	1.0000	
	P=0.025	P=0.060	P=0.085	P=0.067	P=0.233	P=0.025	P=0.001	P=0,000	P=0.000	P=0.000	
NUT21	0.6190	0.4357	0.5440	0.3268	0.7211	0.3636	-0.0926	0 2142	0 1045	0 2771	
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(139)	(170)	
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0.000	P=0.140	P=0.006	P=0.111	P=0.000	
NUT22	0.4460	0.7376	0.5652	0.7110	0.4050	0.8609	0 0975	0 1442	0 11/7	0	
	(138)	(138)	(128)	(138)	(138)	(138)	((38)	0.1443	0.116/	0.1145	
,	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P: 0.000	P=0.128	P=0.046	P=0.086	P=0.091	
NUT23	0.0581	0.0829	0.0377	0.1431	0.0795	0.1452	0.1576	0 3917	0 4222	0.0040	
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(120)	0.2843	
	P=0.249	P=0.167	P≖0.330	P=0.047	P=0.177	P=0.045	P=0.032	P=0.000	P=0.000	P=0.000	
NUT24	0.7753	0.6577	0.7832	0.4918	0.6434	0.6015	-0.0581	0 2202	0 0070	0.0101	
	(138)	(138)	(138)	(136)	(138)	(138)	(138)	(138)	0.0078	0.2606	
	P=0.000	P=0.000	P≈0.000	P=0.000	P=0.000	P= 0.000	P=0.249	P=0.005	P=0.464	P=0.001	
NUT25	0.4683	0.4587	0.4159	0.3449	0.3417	0.3896	-0.0581	0.3910	0 1700	0 4014	
	(138)	(138)	(136)	(138)	(138)	(138)	(138)	(138)	(138)	0.4016	
	P=0.000	P=0.000	P=0.000	F=0.000	P=0.000	P= 0.000	P=0.249	P=0.000	P=0.023	P=0.000	
NUT26	0.8207	0.7165	0.78E7	0.3436	0.6523	0.6089	-0,1228	0.1959	0.0250	0 1744	
	(138)	(138)	(136)	(138)	(138)	(138)	(138)	(138)	(136)	1 1201	
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0.000	P=0.076	P=0.011	P=0.386	P=0.020	
NUT27	-0.0706	-0.0736	-0.0745	-0.0551	-0.0825	-0.1074	-0.0167	-0.1594	-0 1004	-0.1004	
	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(138)	(1304	
	P=0.205	P=0.195	P=0.152	P=0.261	P=0.168	P=0.101	P=0.423	P=0.031	P=0.065	P=0.064	

NUT21 NUT23 NUT24 NUT25 NUT25 NUT26 NUT27 NUT1 0.3744 0.2280 0.6232 0.1784 0.2395 0.0077 0.0077 NUT2 0.4444 0.3062 0.5708 0.188 P=0.002 P=0.133 P=0.464 NUT2 0.4444 0.3062 0.5708 0.4148 0.4618 (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.464 NUT3 0.2657 0.1602 0.4327 0.2276 0.4093 0.2209 -0.1604 NUT4 0.2750 0.1602 0.4327 0.2276 0.4093 0.2209 -0.030 NUT4 0.2760 0.1693 (138)<			PEAP	SON C	ORRELA	TION	COEFFI	CIENTS-
NUT1 0.3744 0.2260 0.6232 0.1784 0.2395 0.0677 0.0077 (138) (138) (138) (138) (138) (138) (138) (138) NUT2 0.4644 0.3662 0.5766 0.4148 0.4844 0.2024 -0.0455 (138) (138) (138) (138) (138) (138) (138) (138) P0.000 P0.000 P0.000 P0.000 P0.000 P0.000 P0.000 P0.000 NUT3 0.2657 0.1602 0.4327 0.2276 0.4093 0.2209 -0.1604 NUT4 0.2780 0.1930 0.5572 0.0506 P0.000 P0.002 P0.0224 P0.473 NUT5 0.7296 0.1939 0.5572 0.05296 0.4295 0.7000 -0.0706 P0.001 P0.003 P=0.278 0.4700 -0.0706 P0.0237 P0.2237 P0.2324 P0.4733 NUT5 0.7296 0.4293 0.2700		NUT21	NUT22	NUT23	NUT24	NUT25	NUT26	NUT27
NUT1 0.3744 0.2260 0.6232 0.1784 0.2395 0.0677 0.0077 NUT2 0.4444 0.3062 0.5766 0.189 (138) (138) (138) NUT2 0.4444 0.3062 0.5766 0.4148 0.4804 0.3934 -0.0455 NUT3 0.2657 0.1002 P=0.000 P=0.000 P=0.000 P=0.000 P=0.024 NUT3 0.2657 0.1022 0.4327 0.2276 0.4093 0.2209 -0.1604 NUT4 0.2770 (138) (138) (138) (138) (138) (138) NUT4 0.2276 0.1930 0.5572 0.0504 P=0.020 P=0.030 P=0.030 NUT5 0.7276 0.3693 0.5270 P=0.220 P=0.220 P=0.237 P=0.207 NUT6 0.2899 0.59748 0.1871 0.4933 0.2423 0.2700 -0.0649 NUT7 0.8007 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000								
NOT1 0.2459 0.2459 0.2459 0.2459 0.2459 0.2459 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2454 0.2455 0.1464 0.2455 0.1464 0.2457 0.2457 0.1402 0.4257 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2457 0.2572 0.4093 0.2259 -0.055 P=0.000	NUTT	0 3744	0 2260	0 4000	0 1704	0 0005	0.0077	0 0077
InstantInstantInstantInstantInstantInstantInstantNUT20.44440.50020.57060.41480.44840.2034-0.0455NUT30.26570.16020.97060.000P=0.000P=0.000P=0.000P=0.000NUT30.26570.16020.43270.22760.40930.2209-0.1604NUT40.27600.19300.55720.0506P=0.000P=0.000P=0.003NUT40.27600.19300.55720.05060.0661-0.05690.0058NUT50.72960.36930.52900.52960.42950.4700-0.0700NUT50.72960.36930.52900.52960.42950.4700-0.0700NUT50.72960.99680.16910.54930.24230.2700-0.0649NUT60.28990.99680.16910.54930.24230.2700-0.0649NUT70.80070.54740.41150.61500.35620.6015-0.0231NUT70.80070.54740.41150.61500.35620.6015-0.0231NUT70.80070.54740.41150.61500.35620.6015-0.0231NUT70.80070.54740.41150.61500.35620.600P=0.000NUT70.80070.54740.41150.61500.35620.6015-0.0231NUT70.80070.54740.41150.61500.35620.6015 <td>NOTI</td> <td>(120)</td> <td>(120)</td> <td>(130)</td> <td>(120)</td> <td>0.2373</td> <td>0.0077</td> <td>0.0077</td>	NOTI	(120)	(120)	(130)	(120)	0.2373	0.0077	0.0077
NUT2 0.4444 0.3062 0.570i 0.4148 0.4644 0.2034 -0.0455 NUT3 0.2657 0.1602 0.306 P=0.000 P=0.0000 P=0.0000<		(138)	1301	1367	1 1387	(138)	(138)	(138)
NUT2 0.4444 0.2042 0.5708 0.4148 0.4881 0.2934 -0.0355 NUT3 0.2457 0.1402 (138)		F=0.000	P=0.004	P=0.000	P=0.018	P=0.002	P=0.153	P=0.464
(138)(138)(138)(138)(138)(138)(138)(138)(138)(138)NUT3 0.2657 0.1602 0.4327 0.2276 0.4093 0.2209 -0.1604 (138)(138)(138)(138)(138)(138)(138)(138)P=0.001P=0.030P=0.000P=0.000P=0.000P=0.000P=0.030NUT4 0.2760 0.1930 0.5572 0.0506 0.0666 -0.0569 0.0058 (138)(138)(138)(138)(138)(138) (138) (138) (138) P=0.001P=0.012P=0.003P=0.278P=0.220P=0.234P=0.473NUT5 0.7296 0.3493 0.5296 0.4295 0.4700 -0.0700 (138) (138)(138)(138)(138) (138) (138) (138) P=0.000P=0.000P=0.000P=0.000P=0.207NUT6 0.2899 0.9948 0.1691 0.5493 0.2720 -0.0649 0.2899 0.9948 0.1691 0.5493 0.2700 -0.0221 NUT7 0.8007 0.5474 0.4115 0.6150 1383 (138) (138) 0.7459 0.3480 0.4293 0.5573 0.4700 -0.0231 0.7459 0.3480 0.4293 0.5573 0.4750 0.593 -0.0515 0.7459 0.3480 0.4293 0.5723 0.3414 0.4771 -0.2258 0.174	NUT2	0.4646	0.3062	0. 5708	0.4148	0.4684	0.2934	-0.0455
P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.299NUT30.2657 (138)0.1602 (138)0.4327 (138)0.2276 (138)0.4093 (138)0.2209 (138)-0.1604 (138)NUT40.2760 (138)0.1930 (138)0.5572 (138)0.0506 (138)0.0661 (138)-0.0559 (138)0.0005 (138)NUT40.2760 (138)0.1930 (138)0.5572 (138)0.0506 (138)0.0661 (138)-0.0559 (138)0.0005 (138)NUT50.7296 (138)0.3693 (138)0.5296 (138)0.4295 (138)0.4700 (138)-0.0700 (138)NUT50.7296 (138)0.3693 (138)0.5296 (138)0.4295 (138)0.4700 (138)-0.0700 (138)NUT60.2899 (138)0.9968 (138)0.1691 (138)0.5493 (138)0.2423 (138)0.2700 (138)-0.0649 (138)NUT70.8007 (138)0.5474 (138)0.4150 (138)0.3562 (138)0.6015 (138)-0.0231 (138)NUT80.7459 (138)0.4800 (138)0.4553 (138)0.4750 (138)0.5693 (138)-0.0515 (138)NUT80.7459 (138)0.3800 (138)0.4520 (138)0.5593 (138)0.4750 (138)0.5693 (138)NUT90.4349 (138)0.3820 (138)0.4520 (138)0.5723 (138)0.4750 (138)0.6753 (138)NUT90.4357 (138)0.4020 (138)0		(138)	(138)	(138)	(138) -	(138)	(138)	(138)
NUT3 0.2657 (138) 0.1602 (138) 0.4227 (138) 0.2276 (138) 0.4093 (138) 0.2209 (138) -0.1603 (138) (138) (138) NUT4 0.2760 (138) 0.1930 (138) 0.5572 (138) 0.0306 (138) (138) (138) (138) (138) (138) (138)		P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0. 000	P=0.298
NUT3 0.2637 (138) 0.1602 (138) 0.4227 (138) 0.2276 (138) 0.093 (138) 0.229 (138) -0.1604 (138)NUT4 0.2760 (138) 0.1930 (138) 0.5572 (138) 0.0306 (138) 0.0661 (138) -0.0567 (138) 0.0058 (138)NUT4 0.2760 (138) 0.1930 (138) 0.5572 (138) 0.0306 (138) 0.0661 (138) -0.0567 (138) 0.0058 (138)NUT5 0.7276 (138) 0.3673 (138) 0.5276 (138) 0.5276 (138) 0.4275 (138) 0.4700 (138) -0.0700 (138)NUT5 0.7276 (138) 0.3673 (138) 0.5276 (138) 0.4275 (138) 0.4700 (138) -0.0700 (138)NUT6 0.2897 (138) 0.9768 (138) 0.1671 (138) 0.5276 (138) 0.4275 (138) 0.4700 (138) -0.0700 (138)NUT6 0.2897 (138) 0.97648 (138) 0.1671 (138) 0.5473 (138) 0.2423 (138) 0.2700 (138) -0.0231 (138)NUT7 0.8007 (138) 0.5474 (138) 0.4150 (138) 0.6015 (138) -0.0231 (138)NUT8 0.7459 (138) 0.3480 (138) 0.4570 (138) 0.6007 (138) 0.4771 (138) -0.0231 NUT8 0.7459 (138) 0.3480 (138) 0.5723 (138) 0.4750 (138) 0.6753 (138) 0.4771 (138) -0.0258 NUT9 0.5335 (138) <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>								
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	NUT3	0.2657	0.1602	0.4327	0.2276	0.4093	0. 2209	-0 1604
P=0.001 P=0.030 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.030 NUT4 0.2760 0.1930 0.5572 0.0506 0.0661 -0.0569 0.0058 P=0.012 P=0.012 P=0.027 P=0.278 P=0.278 P=0.278 P=0.278 P=0.278 NUT5 0.7296 0.3693 0.5290 0.5296 0.4295 0.4700 -0.0700 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 NUT5 0.7296 0.3693 0.5290 0.5294 0.4295 0.4700 -0.0700 NUT6 0.2297 0.9948 0.1891 0.5493 0.2423 0.2700 -0.0449 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000		(138)	(138)	(138)	(138)	(138)	(138)	(138)
NUT4 0.2760 0.1930 0.5572 0.0506 0.0661 -0.0569 0.0058 NUT5 0.2796 0.3493 0.5572 0.0506 0.0661 -0.0569 0.0058 NUT5 0.7276 0.3493 0.5290 0.5294 0.4295 0.4700 -0.0700 NUT5 0.7276 0.3493 0.5290 0.5294 0.4295 0.4700 -0.0700 NUT6 0.2899 0.9948 0.1691 0.5493 0.2423 0.2700 -0.0649 NUT6 0.2899 0.9948 0.1691 0.5493 0.2423 0.2700 -0.0649 NUT6 0.2899 0.9948 0.1691 0.5493 0.2423 0.2700 -0.0449 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.022 NUT7 0.8007 0.5474 0.4115 0.6150 0.3562 0.6015 -0.0231 NUT7 0.8007 0.5474 0.4115 0.6150 0.3562 0.6015 -0.0231 NUT8 0.7459 0.3480 0.4820 0.5		P=0.001	P=0.030	P=0.000	P=0.004	P=0.000	Pr 0 005	R=0.020
NUT4 0.2760 0.1930 0.5572 0.0506 0.0641 -0.0569 0.0058 P=0.001 P=0.012 P=0.003 (138) (138) (138) (138) (138) NUT5 0.7296 0.3693 0.5290 0.5296 0.4295 0.4700 -0.0700 NUT5 0.7296 0.3693 0.5290 0.5296 0.4295 0.4700 -0.0700 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.207 NUT6 0.2699 0.9968 0.1691 0.5493 0.2423 0.2700 -0.0649 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.223 0.2700 -0.0649 NUT7 0.8007 0.5474 0.4115 0.1380 (138) (138) (138) (138) P=0.000						1 -0.000	1-0.000	1-0.030
(138)(138)(138)(138)(138)(138)(138)(138)(138)(138)(138)(138)NUT50.72960.34930.52900.52960.42950.4700-0.0700(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.207NUT60.28990.99680.16910.54930.24230.2700-0.0449(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.013P=0.000P=0.002P=0.01P=0.225NUT70.80070.54740.41150.61500.35620.6015-0.0231(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000NUT80.74590.34800.48300.55730.47500.5693-0.0515(138)(138)(138)(138)(138)(138)(138)(138)NUT90.53530.40200.03250.75940.45870.8765-0.0691(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0	NUT4	0.2760	0.1930	0.5572	0.0506	0.0661	-0.0569	0.0058
P=0.001P=0.012P=0.003P=0.278P=0.220Pt0.234P=0.473NUT50.72960.36930.52900.52960.42950.4700-0.0700(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.207NUT60.28990.99680.16910.54930.24230.2700-0.0649(138)(138)(135)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.013P=0.000P=0.002P=0.022P=0.223NUT70.80070.54740.41150.61500.35620.6015-0.0231(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.394NUT80.74570.34800.48200.55930.347500.5693-0.0515(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.200P=0.200P=0.200NUT90.55350.40200.03250.75940.45870.8765-0.0691NUT00.55350.40200.03250.75940.45870.8207-0.0706P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=		(138)	(138)	(138)	(138)	(138)	(138) •	(138)
NUT5 0.7296 (138) 0.3693 (138) 0.5290 (138) 0.5296 (138) 0.4295 (138) 0.4700 (138) -0.0700 (138)NUT6 0.2899 (138) 0.9488 (138) 0.5493 (138) 0.2423 (138) 0.2700 (138) -0.0649 (138)NUT6 0.2899 (138) 0.9488 (138) 0.5493 (138) 0.2423 (138) 0.2700 (138) -0.0649 (138)NUT7 0.8007 (138) 0.5474 (138) 0.5493 (138) 0.2423 (138) 0.2700 (138) -0.0231 (138)NUT7 0.8007 (138) 0.5474 (138) 0.4115 (138) 0.6150 (138) 0.3562 (138) 0.6015 (138) -0.0231 (138)NUT8 0.7459 (138) 0.3480 (138) 0.4253 (138) 0.5593 (138) 0.4750 (138) 0.5693 (138) -0.0515 (138)NUT9 0.7459 (138) 0.3480 (138) 0.4253 (138) 0.5723 (138) 0.3414 (138) 0.4771 (138)NUT9 0.4349 (138) 0.5383 (138) 0.0850 (138) 0.5723 (138) 0.3414 (138) 0.4771 (138)NUT9 0.5353 (138) 0.4020 (138) 0.3225 (138) 0.7573 (138) 0.4687 (138) 0.8207 (138)NUT10 0.5535 (138) 0.4020 (138) 0.3225 (138) 0.7573 (138) 0.4683 (138) 0.8207 (138) -0.0706 (138)NUT11 0.6190 (138) 0.4460 (138) 0.62		P=0.001	P=0.012	P=0.000	P=0.278	P=0.220	P= 0. 254	P=0.473
NUTS 0.7295 0.3893 0.5290 0.5295 0.4295 0.4295 0.4700 -0.0700 NUT6 (138) (138) (138) (138) (138) (138) (138) (118) (118) NUT6 0.2899 0.9948 0.1691 0.5493 0.2423 0.2700 -0.0649 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000P=0.000P=0.000P=0.000P=0.001P=0.225NUT7 0.6007 0.5474 0.4115 0.6150 0.3562 0.6015 -0.0231 (138) (138) (136) (138) (138) (138) (138) (138) P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.001NUT8 0.7459 0.3480 0.4830 0.5593 0.4750 0.5693 -0.0515 NUT9 0.7459 0.3480 0.4830 0.5573 0.4750 0.5693 -0.0258 NUT9 0.4349 0.5383 0.0850 0.5723 0.3414 0.4771 -0.0258 NUT9 0.4349 0.5383 0.0325 0.7594 0.4587 0.8765 -0.06671 NUT10 0.5535 0.4020 0.0325 0.7594 0.4587 0.8765 -0.06671 NUT11 0.6190 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 NUT12 0.4357 0.7376 0.0829 <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>								
NUT6 (138) (138) (138) (138) (138) (138) (138) (138) (138) NUT6 0.2899 0.9948 0.1891 0.5493 0.2423 0.2700 -0.0649 NUT7 0.8007 0.5474 0.1891 0.5493 0.2423 0.2700 -0.0649 NUT7 0.8007 0.5474 0.4115 0.6150 0.3562 0.6015 -0.0231 NUT7 0.8007 0.5474 0.4115 0.6150 0.3562 0.6015 -0.0231 NUT7 0.8007 0.5474 0.4115 0.6150 0.3562 0.6015 -0.0231 NUT8 0.7457 0.3480 0.44320 0.5593 0.4750 0.5693 -0.0515 NUT8 0.7457 0.3480 0.4830 0.5573 0.4750 0.5693 -0.0515 NUT9 0.4349 0.5383 0.0850 0.5723 0.3414 0.4771 -0.0258 NUT9 0.4349 0.5383 0.0850 0.5723 0.3414 0.4771 -0.0258 NUT10 0.5535 0.4020 0.0325 0.7594 0.4587 0.8765 -0.0691 NUT11 0.4190 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 NUT11 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT13 <t< td=""><td>1015</td><td>0. /296</td><td>0.3693</td><td>0. 5290</td><td>0.5296</td><td>0.4295</td><td>0.4700</td><td>-0.0700</td></t<>	1015	0. /296	0.3693	0. 5290	0.5296	0.4295	0.4700	-0.0700
P=0.000 P=0.0000 P=0.000 P=0.000		(138)	(136)	(138)	(138)	(136)	(138)	(138)
NUT6 0.2899 (138) 0.9948 (138) 0.1691 (136) 0.5493 (138) 0.2423 (138) 0.2700 (138) -0.0049 (138)NUT7 0.8007 (138) 0.5474 (138) 0.4115 (136) 0.6150 (138) 0.3562 (138) 0.6011 (138) -0.0231 (138)NUT7 0.8007 (138) 0.5474 (138) 0.4115 (136) 0.6150 (138) 0.3562 (138) 0.6011 (138) -0.0231 (138)NUT8 0.7459 (138) 0.3480 (138) 0.4830 (138) 0.5593 (138) 0.4750 (138) 0.5693 (138) -0.0515 (138)NUT9 0.7459 (138) 0.3480 (138) 0.4830 (138) 0.5593 (138) 0.4750 (138) 0.5693 (138) -0.0515 (138)NUT9 0.5383 (138) 0.0850 (138) 0.5723 (138) 0.3414 (138) 0.4771 (138) -0.0258 NUT9 0.5535 (138) 0.4020 (138) 0.0325 (138) 0.7573 (138) 0.4587 (138) 0.8765 (138) -0.0691 (138)NUT10 0.5535 (138) 0.4020 (138) 0.0325 (138) 0.7573 (138) 0.4683 (138) 0.8207 (138) -0.0706 (138)NUT11 0.4357 (138) 0.7376 (138) 0.0829 (138) 0.4587 (138) 0.8207 (138) -0.0736 (138)NUT12 0.4357 (138) 0.7376 (138) 0.0829 (138) 0.4587 (138) 0.7573 (138) 0.4683 (138) 0.8207		P=0.000	P=0.000	P≈0.060	P=0.000	P=0.000	P: 0.000	P=0.207
(1, 138) $(1, 138)$ $(1, 135)$ $(1, 138)$ $(1, 138)$ $(1, 138)$ $(1, 138)$ $(1, 138)$ $(1, 138)$ $P=0, 000$ $P=0, 000$ $P=0, 013$ $P=0, 000$ $P=0, 002$ $P=0, 001$ $P=0, 225$ NUT7 $0, 8007$ $0, 5474$ $0, 4115$ $0, 6150$ $0, 3552$ $0, 6015$ $-0, 6231$ $P=0, 000$ $P=0, 374$ NUT8 $0, 7459$ $0, 3480$ $0, 4230$ $0, 5593$ $0, 4750$ $0, 5693$ $-0, 0515$ NUT9 $0, 4349$ $0, 5383$ $0, 0850$ $0, 5723$ $0, 3414$ $0, 4771$ $-0, 0258$ NUT9 $0, 4349$ $0, 5383$ $0, 0850$ $0, 5723$ $0, 3414$ $0, 4771$ $-0, 0258$ NUT10 $0, 5535$ $0, 4020$ $0, 0325$ $0, 7594$ $0, 4587$ $0, 8765$ $-0, 0691$ NUT10 $0, 5535$ $0, 4020$ $0, 0325$ $0, 7753$ $0, 4483$ $0, 8207$ $-0, 0766$ NUT11 $0, 6170$ $0, 4460$ $0, 6581$ $0, 7753$ $0, 4483$ $0, 8207$ $-0, 0766$ NUT12 $0, 4357$ $0, 7376$ $0, 0829$ $0, 6577$ $0, 4587$ $0, 7165$ $-0, 0736$ NUT12 $0, 5446$ $0, 5882$ $0, 6377$ $0, 4587$ $0, 7165$ $-0, 0736$ NUT13 $0, 5446$ $0, 5882$ $0, 6377$ $0, 6377$ $0, 7887$ $-0, 0746$ $P=0, 000$ $P=0, 000$ $P=0, 000$ $P=0, 000$	NUT6	0.2899	0.9968	0.1691	0 5493	0 2423	0 2700	-0.0449
P=0.000P=0.000P=0.013P=0.000P=0.022P=0.001P=0.021NUT70.80070.54740.41150.61500.35620.6015-0.0231(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.394NUT80.74590.34800.45200.55930.47500.5693-0.0515(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.0274NUT90.43490.53830.08500.57230.34140.4771-0.0258(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.382NUT90.43490.53830.08500.57230.34140.4771-0.0258(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.2274NUT100.55350.40200.03250.75940.45870.8765-0.0691NUT110.61900.44600.05810.77530.46830.8207-0.0766(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.247P=0.000P=0.000 <td< td=""><td></td><td>(138)</td><td>(138)</td><td>(135)</td><td>(138)</td><td>(138)</td><td>(133)</td><td></td></td<>		(138)	(138)	(135)	(138)	(138)	(133)	
NUT7 0.8007 0.5474 0.4115 $1-0.000$ $1-0.002$ $p+0.012$ $p+0.223$ NUT7 (138) (138) (138) (138) (138) (138) (138) (138) (138) P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000NUT8 0.7459 0.3480 0.4230 0.5593 0.4750 0.5693 -0.0515 (138) (138) (138) (138) (138) (138) (138) (138) $P=0.000$ $P=0.000$ $P=0.000$ $P=0.000$ $P=0.000$ $P=0.274$ NUT9 0.4349 0.5383 0.0850 0.5723 0.3414 0.4771 -0.0258 (138) (138) (138) (138) (138) (138) (128) $P=0.000$ <td></td> <td>P≈0,000</td> <td>P=0.000</td> <td>P=0 017</td> <td>P=0 000</td> <td>R=0.002</td> <td>R=0.001</td> <td>1. 138)</td>		P≈0,000	P=0.000	P=0 017	P=0 000	R=0.002	R=0.001	1. 138)
NUT70.8007 (138)0.5474 (138)0.4115 (138)0.6150 (138)0.3562 (138)0.6015 (138)-0.0231 (138)NUT80.7459 (138)0.3480 (138)0.4830 (138)0.5593 (138)0.4750 (138)0.5693 (138)-0.0515 (138)NUT80.7459 (138)0.3480 (138)0.4830 (138)0.5593 (138)0.4750 (138)0.5693 (138)-0.0515 (138)NUT90.4349 (138)0.5383 (138)0.0850 (138)0.5723 (138)0.3414 (138)0.4771 (138)-0.0258 (138)NUT90.4349 (138)0.5383 (138)0.0850 (138)0.5723 (138)0.3414 (138)0.4771 (138)-0.0258 (138)NUT90.5535 (138)0.4020 (138)0.0325 (138)0.7594 (138)0.4587 (138)0.8765 (138)-0.0691 (138)NUT100.5535 (138)0.4020 (138)0.0325 (138)0.7594 (138)0.4683 (138)0.8207 (138)-0.0706 (138)NUT110.6190 (138)0.4460 (138)0.0581 (138)0.7753 (138)0.4683 (138)0.8207 (138)-0.0706 (138)NUT120.4357 (138)0.7376 (138)0.0829 (138)0.4577 (138)0.4587 (138)0.7165 (138)-0.0736 (138)NUT130.5446 (138)0.5882 (138)0.6377 (138)0.7827 (138)0.7867 (138)-0.0746 (138)NUT130.5446 (138)0.5882 (138)0.6377					1 0.000	1-0.002	1-0.001	F=0.225
(138)(138)(136)(138) <th< td=""><td>NUT7</td><td>0.8007</td><td>0.5474</td><td>0.4115</td><td>0.6150</td><td>0.3562</td><td>0.6015</td><td>-0.0231</td></th<>	NUT7	0.8007	0.5474	0.4115	0.6150	0.3562	0.6015	-0.0231
P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.000P=0.394NUTB0.74590.34800.48300.55930.47500.5693-0.0515(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.000P=0.000P=0.000P=0.274NUT90.43490.53830.08500.57230.34140.4771-0.0258(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.161P=0.000P=0.000P=0.362NUT100.55350.40200.03250.75940.45870.8765-0.0691(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.353P=0.000P=0.000P=0.210NUT110.61900.44600.05810.77530.46830.8207-0.0706(138)(138)(138)(138)(138)(138)(138)(138)P=0.000P=0.000P=0.247P=0.000P=0.000P=0.205NUT120.43570.73760.08290.65770.45870.7165-0.0736NUT130.54460.58820.63770.78220.41990.7887-0.0746NUT130.54460.58820.63770.78220.41990.7887-0.0746P=0.000P=0.000P=0.000P=0.000P=0.000P=0.167		(138)	(138)	(135.)	(138)	(138)	(138)	(138)
NUTB 0.7459 (138) 0.3480 (138) 0.4830 (138) 0.5593 (138) 0.4750 (138) 0.5693 (138) -0.0515 (138)NUT9 0.4349 (138) 0.5383 (138) 0.0850 (138) 0.5723 (138) 0.3414 (138) 0.4771 (138) -0.0258 (138)NUT9 0.4349 (138) 0.5383 (138) 0.0850 (138) 0.5723 (138) 0.3414 (138) 0.4771 (138) -0.0258 (138)NUT9 0.4349 (138) 0.5383 (138) 0.0850 (138) 0.5723 (138) 0.3414 (138) 0.4771 (138) -0.0258 (138)NUT10 0.5535 (138) 0.4020 (138) 0.0325 (138) 0.7594 (138) 0.4587 (138) 0.8765 (138) -0.0691 (138)NUT10 0.5535 (138) 0.4020 (138) 0.0325 (138) 0.7594 (138) 0.4587 (138) 0.8765 (138) -0.0691 (138)NUT11 0.6190 (138) 0.4460 (138) 0.7533 (138) 0.4683 (138) 0.8207 (138) -0.0706 (138)NUT11 0.6190 (138) 0.7376 (138) 0.7753 (138) 0.4683 (138) 0.8207 (138) -0.0706 (138)NUT12 0.4357 (138) 0.7376 (138) 0.0829 (138) 0.4577 (138) 0.4587 (138) 0.7165 (138)NUT13 0.5446 (138) 0.6377 (138) 0.7822 (138) 0.7887 (138) 0.7887 (138) -0.0746 (138)NUT13 <t< td=""><td></td><td>P=0.000</td><td>P=0.000</td><td>P=0.000</td><td>P=0.000</td><td>P=0.000</td><td>P= 0.000</td><td>P=0.394</td></t<>		P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0.000	P=0.394
NUT0 0.3430 0.3430 0.3333 0.4730 0.3543 0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.025 0.3414 0.4771 -0.0258 (138) (1	NUTR	0 7450	0 3490	0 4520	0 5500	0 4750	0 5/00	0.0515
NUT9 (-138) (-138) (-138) (-138) (-138) (-138) NUT9 (-138) (-138) (-138) (-138) (-138) (-138) (-138) P=0.000P=0.000P=0.000P=0.000P=0.000P=0.274NUT9 (-138) (-138) (-138) (-138) (-138) (-138) P=0.000P=0.000P=0.161P=0.000P=0.000P=0.200P=0.000P=0.000P=0.161P=0.000P=0.000P=0.382NUT10 (-5535) (-138) (-138) (-138) (-138) (-138) (-138) (-138) (-138) (-138) P=0.000P=0.000P=0.161P=0.000P=0.000P=0.382NUT10 (-5535) (-0.000) (-138)	NOTO	(138)	(120)	(120)	0. 5543	0.4750	0. 5693	-0.0515
NUT90.4349 (138)0.5383 (138)0.0850 (138)0.5723 (138)0.3414 (138)0.4771 (138) -0.0258 (138)NUT90.4349 (138)0.5383 (138)0.0850 (138)0.5723 (138)0.3414 (138)0.4771 (138) -0.0258 (138)NUT100.5535 (138)0.4020 (138)0.0325 (138)0.7594 (138)0.4587 (138)0.8765 (138) -0.0691 (138)NUT100.5535 (138)0.4020 (138)0.0325 (138)0.7594 (138)0.4587 (138)0.8765 (138) -0.0691 (138)NUT110.4517 (138)0.4460 (138)0.6581 (138)0.7753 (138)0.4683 (138)0.8207 (138) -0.0706 (138)NUT110.4470 (138)0.4460 (138)0.6581 (138)0.7753 (138)0.4683 (138)0.8207 (138) -0.0706 (138)NUT110.4470 (138)0.4460 (138)0.6581 (138)0.7753 (138)0.4683 (138)0.8207 (138) -0.0706 (138)NUT120.4357 (138)0.7376 (138)0.6229 (138)0.6577 (1438)0.4587 (138)0.7165 (138)NUT130.5446 (138)0.5882 (138)0.6377 (138)0.7487 (138) -0.0746 (138)NUT130.5446 (138)0.5882 (138)0.6377 (138)0.7822 (138)0.4199 (138)0.7887 (138)NUT130.5446 (138)0.5882 (138)0.6377 (138)0.7827 (138)0.7887 (R=0.000	R=0.000		(138)	(138)	(138)	(138)
NUT9 0.4349 0.5383 0.0850 0.5723 0.3414 0.4771 -0.0258 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000P=0.000P=0.161P=0.000P=0.000P=0.000P=0.382NUT10 0.5535 0.4020 0.0325 0.7594 0.4587 0.8765 -0.0691 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000P=0.000P=0.000P=0.000P=0.000P=0.210NUT11 0.6190 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000P=0.000P=0.247P=0.000P=0.000P=0.205NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT13 0.5446 0.5882 0.0377 0.7822 0.4199 0.7887 -0.0746 N		-0.000	F=0.000	F=0.000	F=0.000	P=0.000	P10.000	P=0.2/4
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	NUT9	0.4349	0. 5383	0.0850	0.5723	0.3414	0.4771	-0.0258
P=0.000 P=0.000 P=0.161 P=0.000 P=0.000 P=0.000 P=0.382 NUT10 0.5535 0.4020 0.0325 0.7594 0.4587 0.8765 -0.0691 (138) (138) (132) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.353 P=0.000 P=0.000 P=0.210 NUT11 0.6190 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.247 P=0.000 P=0.000 P=0.205 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT13 0.5446 0.5882 0.6377 0.7827 0.7887 -0.0746 NUT13 0.5446 0.5882 0.6377 0.7827 0.4199 0.7887 -0.0746 <t< td=""><td></td><td>(138)</td><td>(138)</td><td>(138)</td><td>(138)</td><td>(138)</td><td>(138)</td><td>(138)</td></t<>		(138)	(138)	(138)	(138)	(138)	(138)	(138)
NUT10 0.5535 0.4020 0.0325 0.7594 0.4587 0.8765 -0.0691 NUT10 138 (138)		P=0.000	P=0.000	P=0.161	P=0.000	P=0.000	P=0.000	P=0.382
NOTIO 0.3333 0.4020 0.0325 0.754 0.4587 0.8765 -0.0671 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.353 P=0.000 P=0.000 P=0.000 P=0.210 NUT11 0.6190 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.247 P=0.000 P=0.000 P=0.205 NUT12 0.4357 0.7376 0.0827 0.6577 0.4587 0.7165 -0.0736 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.167 P=0.000 P=0.000 P=0.195 NUT13 0.5446 0.5882 0.6377 0.7822 0.4197 0.7887 -0.0746 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000	NUTIO	0 8505	0 4000	0.0005				
NUT11 0.6170 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 NUT11 0.6170 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.195 NUT13 0.5446 0.5882 0.037" 0.7822 0.4199 0.7887 -0.0746 NUT13 0.5446 0.5882 0.037" 0.7822 0.4199 0.7887 -0.0746 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.192 <td>NUTTO</td> <td>0. 5535</td> <td>0.4020</td> <td>0.0325</td> <td>0.7594</td> <td>0.4587</td> <td>0.8765</td> <td>-0.0691</td>	NUTTO	0. 5535	0.4020	0.0325	0.7594	0.4587	0.8765	-0.0691
P=0.000 P=0.000 P=0.333 P=0.000 P=0.000 P=0.210 NUT11 0.6190 0.4460 0.0581 0.7753 0.4683 0.8207 -0.0706 (138) (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.247 P=0.000 P=0.000 P=0.000 P=0.205 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 NUT13 0.5446 0.5882 0.0377 0.7822 0.4199 0.7887 -0.0746 NUT13 0.5446 0.5882 0.0377 0.7822 0.4199 0.7887 -0.0746 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.0746		1387	(138)	(138)	(138)	(138)	(138)	(138)
NUT11 0.6170 (138) 0.4460 (138) 0.0581 (138) 0.7753 (138) 0.4683 (138) 0.8207 (138) -0.0706 (138) NUT12 0.4357 (138) 0.7376 (138) 0.0829 P=0.000 0.6577 P=0.000 0.4587 P=0.000 0.7165 P=0.000 -0.0736 P=0.205 NUT12 0.4357 (138) 0.7376 (138) 0.0829 (138) 0.6577 (138) 0.4587 (138) 0.7165 (138) -0.0736 (138) NUT13 0.5446 (138) 0.5882 (138) 0.0377 0.7822 (138) 0.4199 (138) 0.7887 (138) -0.0746 (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.127		P=0.000	P=0.000	P=0.353	P=0.000	P=0.000	P= 0.000	P=0.210
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	NUT11	0.6190	0.4460	0.0581	0.7753	0.4683	0.8207	-0 0706
P=0.000 P=0.000 P=0.247 P=0.000 P=0.000 P=0.205 NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.167 P=0.000 P=0.000 P=0.195 NUT13 0.5446 0.5882 0.6377 0.7822 0.4199 0.7887 -0.0746 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.020 P=0.175		(138)	(138)	(138)	(138)	(138)	(138)	(138)
NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.167 P=0.000 P=0.000 P=0.000 P=0.000 P=0.195 NUT13 0.5446 0.5882 0.037" 0.7822 0.4199 0.7887 -0.0746 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.192		P=0.000	P=0.000	P=0. 247	P=0.000	P=0.000	P=0.000	P=0.205
NUT12 0.4357 0.7376 0.0829 0.6577 0.4587 0.7165 -0.0736 (138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.167 P=0.000 P=0.000 P=0.000 P=0.195 NUT13 0.5446 0.5882 0.0377 0.7822 0.4199 0.7887 -0.0746 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.000 P=0.000 P=0.000 P=0.175						1-01-003	1-01000	1-0.205
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	NUT12	0.4357	0.7376	0.0829	0.6577	0.4587	0.7165	-0.0736
P=0.000 P=0.000 P=0.167 P=0.000 P=0.000 P=0.000 P=0.195 NUT13 0.5446 0.5882 0.637? 0.7822 0.4199 0.7887 -0.0746 (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.330 P=0.000 P=0.000 P=0.192		(138)	(138)	(138)	(138)	(138)	(138)	(138)
NUT13 0.5446 0.5882 0.637? 0.7822 0.4199 0.7887 -0.0746 (138)		P=0.000	P=0.000	P=0.167	P=0.000	P=0.000	P= 0.000	P=0.195
NUT13 0.5446 0.5882 0.037? 0.7822 0.4199 0.7887 -0.0746 (138)								
(138) (138) (138) (138) (138) (138) (138) (138) P=0.000 P=0.000 P=0.330 P=0.000 P=0.000 P=0.000 P=0.192	NUT13	0. 5446	0.5882	0.0377	0.7822	0.4199	0.7887	-0.0746
P=0.000 P=0.000 P=0.330 P=0.000 P=0.000 P+0.000 P=0.192		(138)	(138)	(138)	(138)	(138)	(138)	(138)
		P=0.000	P=0.000	P=0. 330	P=0.000	P=0.000	P=0.000	P=0.192

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NUT15	0.7211	0.4050	0.0795	0.6434	0.3417	0.6523	-0.0825
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.000	P=0.177	P=0.000	P=0.000	P= 0.000	P=0.168
NUT16	0.3636	0.8609	0.1452	0.6016	0.3876	0.6089	-0,1094
	(138)	(138)	(138)	(138)	(136)	(138)	(138)
	P=0.000	P=0.000	P=0.045	P=0.000	P=0.000	P=0.000	P=0.101
NUT17	-0.0926	0.0975	0.1576	-0.0581	-0.0581	-0.1226	-0.0167
	(138)	(138)	(136)	(138)	(138)	(138)	(138)
	P=0.140	P=0.128	P=0.032	P=0.249	P=0.249	P: 0.076	P=0.423
NUT18	0.2142	0.1443	0.3917	0.2203	0.3910	0.1959	-0.1596
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.006	P=0.046	P=0.000	P=0.005	P=0.000	P=0.011	P=0.031
NUT19	0.1048	0.1167	0.4333	0.0078	0.1700	0.0250	-0.1294
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0, 111	P=0.086	P=0.000	P=0.464	P=0.023	P= 0.386	P=0.065
NUT20	0.2774	0.1145	0. 2843	0 2606	0 4016	0 1744	-0 1204
	(138)	(138)	(138)	(130)	(170)	0.1740	-0.1304
	P=0.000	P≈0.091	P=0.000	P=0.001	P=0.000	P=0.020	P=0.064
NUT21	1.0000	0.2945	0.3172	0. 5234	0.3026	0 4124	-0.0050
	(138)	(138)	(138)	(138)	(138)	(138)	(128)
	P=0.000	P=0.000	P=0.000	P=0.000	P=0.000	P= 0. 000	P=0.477
NUT22	0.2945	1.0000	0.1905	0.5511	0.2391	0.2739	-0.0753
	(138)	(138)	(138)	(138)	(13E)	(136)	(138)
	P=0.000	P=0.000	P=0.013	P=0.000	P=0.002	P=0.001	P=0.190
NUT23	0.3172	0. 1905	1.0000	0.1000	0.1777	0.0709	0.0476
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.013	P=0.000	P=0.122	P=0.019	P: 0. 204	P=0.290
NUT24	0. 5234	0.5511	0.1000	1.0000	0.4769	0.6469	-0.0399
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.000	P=0.122	P=0.000	P=0.000	P=0.000	P=0.321
NUT25	0.3026	0.2391	0.1777	0.4769	1.0000	0.4531	-0.0278
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.002	P=0.017	P=0.000	P=0.000	P= 0. 000	P=0.373
NUT26	0.4124	0.2739	0.0709	0.6469	0.4531	1.0000	-0.0656
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.000	P=0.001	P=0.204	P=0.000	P=0.000	P=0.000	P=0.222
NUT27	-0.0050	-0.0753	0.0476	-0.0399	-0.0278	-0.0656	1.0000
	(138)	(138)	(138)	(138)	(138)	(138)	(138)
	P=0.477	P=0.190	P=0.290	P=0.321	P=0.373	P=0. 222	P=0.000

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(COEFFICIENT / (CASES) / SIGNIFICANCE) (A VALUE OF 97.0000 IS PRINTED IF A COEFFICIENT CANNOT BE COMPUTED)

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Appendix F. A Complete Data File

VARIABLE	FORMAT	RECORD	COLUMNS	DESCRIPTION
GROUP PERIOD OLDNO NEWNO AGE	F 1. 0 F 1. 0 F 3. 0 F 2. 0 F 2. 0 F 4 1	1 1 1	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	GROUP # PERIOD # SUBJECT # SUBJECT #
WEIGHT	F 5. 1	1	16- 20	
GRAVID	F 1. 0	4	23- 23	# OF CHILDREN
SUPP	F 1. 0	1	27- 27	USE OF SUPPLEMENTS W/PA
CUNTR	F 1, U	1	31- 31	WEIGHT REDUCTION DIFT
WIRED DI OOD	F 1. 0		38- 44	BLOOD PA LEVEL (ng/m])
PLASMA	F 7. 3	-	46- 52	PLASMA PA LEVEL(ng/m])
CR	F 7. 2	1	53- 59	DAILY CREATININE(mg/day)
PAD	F 5. 2		63- 67	URINARY PA(mg/day)
PACR	F 5. 2	1	71- 75	URINARY PA/CR(mg/g)
PREM	F 4. 2	6. 10	77- 30	PA IN FOREMILK(g/ml)
POSTM	F 4. 2	1	84- 87	PA IN HINDMILK(g/ml)
NUTI	F 8. 2		89- 95	KCALORIES
NUT2 ·	F 8. 2		7/- 104	PROTEIN(g)
NULS	F 8. 2		1927 112	CAPBOHYDRATE(a)
	F 6. 4		121- 128	CALCIUM(mg)
NUTA	. 0. 1		109- 136	IRON (mg)
NUTZ	F 8 2	-	137- 144	MAGNESIUM(mg)
NUTR	F 8. 2		145- 152	PHOSPHORUS (mg)
NUT9	F 9. 2		153~ 161	VITAMIN A(IU)
NUTIO	F 9. 2	2	162- 170	THIAMIN(mg)
NUT11	F 8. 2	×	171- 178	RIBOFLAVIN(mg)
NUT12	F 8. 2	1	179- 186	NIACIN(mg)
NUT13	F 8. 2	1	187- 194	VITAMIN B6(mg)
NUT14	F 8, 2	1	195- 202	VITAMIN C(mg)
NUT15	F 8. 2	2	203- 210	FOLATE(g)
NUTIE	F 8. 2	÷	211- 213	ADDED SUGAR(g)
NUTIO	F 7. Z	÷ 1	228- 235	TOTAL SATURATED F.A. (mg)
NUT10	F 8 2	- -	236- 243	TOTAL POLY UNSA F.A. (mg)
NUT20	F 8, 2	1	244- 251	TOTAL MONO UNSA F.A. (mg)
NUT21	F 8. 2	1	252- 259	POTASSIUM(mg)
NUT22	F 8. 2	1	260- 267	IRON(NEW)(mg)
NUT23	F 8. 2	1	268- 275	SODIUM (mg)
NUT24	F 8. 2	4.0	276- 283	
NUT25	F8.2	1	284- 291	CHULESIERUL(MG)
NUT26	F 8. 2	1	292- 299	PANTOTHENIC ACID(mg)
NUTE7	F 8. 2	1	300- 307	ALLUHUL(g)

\$ SET TERM/WIDTH=75 \$ IY ALL. DAT 11101 131. 66.0150.0 2. 0. 0. 0. 588.00 118.80 694.05 2.54 3.66 1787.00 79.20 47.55 262.67 997.76 9.69 280.49 1286.9 1.14 1.53 22.81 1.40 2.52 176.49 247.57 71.2 4 12898.79 6 14.44 5.93 18.26 1993.02 10.60 1642.24 9.97 189.99 3.95 0.00 12101 131. 66.0150.0 2. 0. 0. 0. 519.33 158.84 1471.66 68.85 76.18 130.18 638.99 9.84 206.95 934.1 2 17051.67 1.11 1.23 22.42 1.39 2.33 141.44 156.84 22.7 2 27.99 13.00 28.53 1821.52 10.70 1662.42 8.82 241.54 3.70 0.00 13101 131. 66.0150.0 2. 0. 0. 0. 382.56 92.80 823.47 5.09 6.18 1712.27 67.78 99.40 128.03 469.59 12.56 270.07 1060.5 0 8373.12 1.62 1.42 27.13 1.70 1.81 123.88 310.90 7.3 1 37.30 28.89 26.36 2598.61 10.80 2955.33 8.09 468.13 5.56 10.23 11102 226. 70.5135.0 1. 0. 0. 0. 420.00 89.20 790.64 2.32 2.94
 1874.87
 91.05
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 1126.96
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 8732.27
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 574.73
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 4 28.42 12.34 32.16 2676.80 14.29 2190.36 9.11 535.93 5.09 0.00 12102 226. 70.5135.0 1. 0. 0. 0. 498.00 101.67 2102.25 84.50 94.95 234.10 1360.75 12.32 306.63 1571.8 1.73 2.25 19.11 1.14 5.44 162.50 267.15 19.9 2 4111.79 4 36.01 13.02 39.39 3106.54 13.42 4001.57 8.89 498.35 6.77 0.00 13102 226. 70.5135.0 1. 0. 0. 0. 701.77 93.05 864.16 4.46 5.18
 2118.58
 87.68
 82.45
 260.25
 1327.27
 22.67
 264.17
 1532.5

 2.03
 3.04
 139.77
 3.01
 8.61
 54.00
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VITA

Won O. Song

Candidate for the Degree of

Doctor of Philosophy

Dissertation: Longitudinal Pantothenic Acid Status of Pregnant and Lactating Women

Major Field: Nutrition and Food Sciences

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