DIETARY FOLATE AND

SERUM FOLATE

LEVELS

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1986

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ACKNOWLEDGEMENTS

I express my sincere appreciation to Dr. Esther Winterfeldt for her encouragement, support and assistance during my graduate program. I also extend my appreciation to the other members of my committee: Dr. Fred Owens, for his encouragement and valuable advice and assistance in the conceptualization, data analysis, and writing of this thesis; Dr. Marguerite Scruggs and Dr. Calvin Beames for their support and assistance. I also thank Dr. Mary Alice Kenney for her guidance in planning my research and supporting my graduate program.

Special thanks go to my colleagues Kathy Yadrick and Andrea Arquitt who have been a continuous source of ideas, encouragement, technical assistance and friendship during the course of this research.

I reserve my warmest appreciation for my family: my mother, Shirley Foster, my sister, Jane, and my brother, Paul, for never doubting I could do it and my husband, Bob, for making the accomplishment worthwhile.

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CHAPTER I

INTRODUCTION

Folacin is a component of coenzymes which transport single carbon units. This vitamin, a derivative of pteroylglutamic acid, functions in the synthesis of the purine and pyrimidine bases of the nucleic acids in DNA and RNA as well as in amino acid metabolism. Insufficient folacin impairs nucleotide synthesis and cell division and alters the synthesis of other proteins (National Research Council, 1980; Colman, 1981). Folacin is one of the nutrients essential for hematopoiesis and lowered hematocrit and hemoglobin concentration can result from its deficiency (Bailey, et al, 1980).

Intakes of folacin calculated from food consumption surveys of populations have consistently fallen below recommended intake (Hoppner et al, 1972; Hoppner et al, 1977; Bates et al 1982; Poh Tan et al, 1984) while measured body stores of folate have not been correspondingly low. Participants in the 1977 National Research Council Workshop on Human Folate Requirements (Herbert, 1977) recommended lowering the U.S. folate Recommended Dietary Allowance for adults from 400 mcg per day to 300 mcg per day.

Until recently folate contents of individual foods have been underestimated. It was recognized from the outset that the 1951 U.S.D.A. Handbook No. 29 (Toepfer et al, 1951) provided unrealistically low food folate values due to failure to preserve the folate activity of samples with ascorbic acid during the assay. More recent and complete analyses of folate in foods are now available (Hoppner et al, 1972; Santini and Corcino, 1974; Hoppner et al, 1977; Perloff and Butrum, 1977; Spring et al, 1979). At this time there is enough accurate data on the folate content of individual foods to expect calculated and assayed dietary values to be in reasonably close agreement. Since expansion of the data base for the folate content of foods, little research has been directed toward quantifying the relationship between dietary folate and blood folate levels in individuals.

Folate deficiency is defined as tissue depletion of the vitamin. Deficiency is demonstrated by below normal tissue levels or subnormal function of folate-dependent enzyme systems (Herbert, 1981). Serum folate is the most rapidly changing blood parameter in response to dietary folate and is indicative of recent folate intake. Red blood cell folate more accurately reflects body stores which mirror nutritional status. Serum folate reached deficient concentrations, below three ng/ml (Sauberlich, 1977), as early as three weeks after the onset of dietary folate deprivation (Herbert, 1962b). Although concentration of

serum folate alone is not an accurate indicator of body stores, continued low concentration of serum folate over time would be expected to be associated eventually with tissue depletion and megaloblastic anemia (Sauberlich, 1977).

Serum folate concentration fell significantly in a small number of subjects after only one week on a folate deficient diet (Herbert, 1962b). Eichner and Hillman (1973) found a mean drop in serum folate of nine percent in one day and 30 percent in six days in six subjects consuming a diet providing five mcg total folate per day compared with a maintenance intake of 150-200 mcg folate daily. In a recent study of sixty adult women (Rhode et al, 1983) mean serum folate of adult women decreased to 62 percent of its initial level in two weeks on a self-selected folate-restricted diet calculated to provide 159 mcg total folate per day. Supplementation of this diet with 100 mcg folate per day for seven subsequent weeks increased mean serum folate significantly.

Serum folate is the biochemical parameter most frequently measured as an indicator of folate status. The serum assay is simpler and the results are more reproducible than those of the red blood cell folate assay. In large scale nutrition surveys a measurement of serum folate, sometimes fasting and sometimes not, with or without a red blood cell folate measurement, is frequently used as an indicator of the folate status of population groups (Ten

State Nutrition Survey, 1972; Nutrition Canada, 1973; Lan, 1982). A diet history, food frequency or one or more 24 hour dietary recalls is often obtained at the time blood is drawn.

Research to date indicates serum folate concentrations in humans consuming self-selected diets fluctuate daily and that serum folate concentration can rise and fall in a few days in response to dietary folate depletion or supplementation. Response of serum folate to depletion or supplementation also may be influenced by initial serum folate concentration. Data on daily variation of serum folate concentration within individuals and betweeen individuals in response to self-selected, low folate and high folate diets is lacking.

Purpose and Objectives

The purpose of this study was to determine the effects of short term changes in folate intake on serum folate (SF) and red blood cell folate (RBCF) of adult women. The following objectives were formulated:

1. To determine the day-to-day changes in fasting SF concentration within individuals and between individuals consuming a non-restricted self-selected diet.

2. To determine the day-to-day change in fasting SF concentration within individuals and between individuals consuming a low folate (calculated at 50 mcg total folate

daily) diet for one week with and without folate supplementation (400 mcg/day added folate daily).

3. To determine the correlation between initial SF and SF in response to a low folate or a high folate diet.

4. To determine the difference between users (OCA) and non-users (non-OCA) of oral contraceptive agents in SF and RBCF.

5. To determine the usefulness of a Food Folate Checklist which considers only foods which are rich in folic acid to estimate daily folate intake.

Hypotheses

1. Individual mean fasting SF concentration will be positively associated with individual mean daily folate intake among adult women consuming a self-selected diet during one week.

2. Folate intake restriction to 50 mcg total folate per day will lower fasting SF concentration in one week or less among a sample of adult women.

3. The decrease in fasting SF concentration in one week on a low folate diet will be inversely proportional to initial fasting SF concentration.

4. A folate supplement of 400 mcg total folate per day will increase fasting SF concentration within one week in adult women.

5. The increase in fasting SF concentration on a high folate diet will be inversely proportional to initial fasting SF concentration after consuming a self-selected diet or a low folate diet for one week among a sample of adult women.

6. Order of diet treatment will not alter the response in SF or RBCF to a low and high folate diet.

7. There will be a difference between SF and RBCF of OCA and non-OCA on the self-selected diet as well as on the low and high folate diets.

8. Daily total folate intake calculated from a Food Folate Checklist including only significant food sources of folic acid will correlate positively with folate intake calculated from a 24-hour dietary record.

Assumptions and Limitations

1. It is assumed that dietary records represent actual food intake of subjects.

2. It is assumed that the calculated total folate content of the experimental diet accurately represents the actual total folate content.

3. It is assumed that subjects adhered to the prescribed dietary and blood collection protocol.

4. Application of the findings may be limited because the sample was a small group of volunteers and not necessarily representative of a larger population.

Format of Dissertation

The two major aspects of this experiment were each prepared as individual manuscripts for publication in the most applicable journal. Chapter III was written according to the Guide for Authors for the Journal of Nutrition. Chapter IV followed the Guidelines for Authors for the Journal of the American Dietetic Association.

Chapter II

REVIEW OF LITERATURE

Absorption and Metabolism of Naturally Occurring Folates

Naturally occurring folates are derived from folic acid. Folic acid is structurally composed of a pteridine moiety, p-amino-benzoic acid and one glutamyl residue. Folic acid itself is not present in biological materials. The natural folates are modified at one of three locations. These modifications include 1) reduction of the pteridine ring to 7-8-dihydrofolate or 5-6-7-8-tetrahydrofolate 2) presence of any one of six one-carbon units at the N-5 or N-10 position or between the two (5-methyl, 5-formyl, 10-formyl, 5,10-methylene, 5,10-methenyl and 5-formimino) and 3) increase in the number of glutamate residues to as many as eight attached by gamma-peptide linkages (Colman, 1981).

Dietary folates are primarily reduced and conjugated poly-gamma-glutamate derivatives with a variety of substitutions on the pteridine ring (Olinger et al, 1973; Tyerman et al, 1977). Over 90 percent of dietary folates are pteroylpolyglutamates. The most common of these is 5-methyl tetrahydropolyglutamate. The other ten percent of

food folates are primarily 10-formyl tetrahydromonoglutamate (Halsted, 1980). The rate of absorption of dietary folate is dependent upon glutamate chain length as well as the one-carbon substitutions at the 5- and 10- positions (Brown et al, 1973). Acidity and alkalinity are food characteristics which negatively affect folate absorption by interfering with polyglutamate hydrolysis and mucosal transport respectively (Colman, 1981). The composition of dietary folates from foods (Retief and Phil, 1969; Tamura and Stokstad, 1973; Abad and Gregory, 1985) and synthetic folates (Brown et al 1973) has been found to influence folate bioavailability. The absorption of pure folate polyglutamates is comparable to that of monoglutamate. The conjugated forms in foods, however, may be less well absorbed due to the presence of natural conjugase inhibitors (Stokstad et al, 1977). The term "free" dietary folate refers to monoglutamate food forms which can be measured by L. casei activity without prior conjugase treatment. "Total" dietary folate refers to all mono- and polyglutamate forms which respond to L. casei after conjugase treatment (Butterworth et al, 1969) and is the basis for the RDA for folic acid which is 400 mcg total folate (National Research Council, 1980).

Folate Absorption

The hydrolysis of folate polyglutamates to monoglutamates must precede absorption (Colman, 1981). The

mammalian intestinal conjugase or conjugases responsible for this function have not been specifically characterized or named at present. Evidence indicates both a gamma-glutamylcarboxypeptidase which hydrolyzes glutamic acid residues stepwise from the carboxyl end of the molecule and an endopeptidase (Rosenberg, 1977) may be involved. Mucosal deconjugation of polyglutamates was observed in rats (Rosenberg et al, 1969) when polyglutamyl folate from yeast was incubated on the mucosal side of everted gut sacs and monoglutamyl folate was recovered on the serosal side after 60 minutes. When human mucosal homogenates were the source of enzymes, the result was similar. Butterworth et al (1969) administered oral ^{14}C-labeled polyglutamates to humans and observed that serum label and folate activity rose in proportion to intake only when the label was positioned on the first glutamyl unit. This demonstrated that deconjugation to monoglutamate preceded absorption.

Perry and Chanarin (1970) demonstrated in humans that orally administered dihydrofolate is reduced to tetrahydrofolate and methylated in the gut during absorption and that already-reduced tetrahydrofolate is methylated before absorption. Oxidized PGA is reduced to the dihydro-form at only about one-tenth the rate that dihydro- is reduced to tetrahydrofolate. The role of the enzyme dihydrofolate reductase in folate reduction in the rat gut was demonstrated by inhibition of folate absorption by methotrexate, a dihydrofolate reductase binder (Burgen and

Goldberg, 1962). The reduction process may be saturable. Reduced forms of monoglutamate are methylated during absorption. Since the enzymes which add one-carbon units to the pteroyl moiety require reduced folates, pteroylglutamic acid is only partially methylated during transport (Rosenberg and Godwin, 1971).

Absorption of folate occurs almost entirely in the proximal jejunum (Hepner et al, 1968) as evidenced by greatly decreased absorption among celiac patients with proximal jejunal malabsorption. Absorption was found to be an active saturable process in rats (Burgen and Goldberg, 1962) and to proceed against a 30-fold concentration gradient in humans (Hepner et al, 1968).

The transport form of folate is pteroylmonoglutamate. The functional coenzyme forms are pteroylpolyglutamates. Polyglutamyl forms do not readily cross cell membranes so are retained by the tissues (Tyerman et al, 1977). Identification of folate metabolites is carried out using labeled tracer doses of the vitamin which are differentiated by ion-exchange and gel chromatography together with differential microbiological assay before and after conjugase treatment or by comparing elution profiles of metabolites with known folate standards.

Folate undergoes enterohepatic circulation. Methyltetrahydrofolate passes from the mucosal cell into the systemic circulation. The liver receives methylated folate from the portal vein as well as some demethylated folate

from non-hepatic tissue. The nonmethylated folates are methylated in the liver and, along with the methylated folates absorbed, either are converted to polyglutamates for the hepatic folate pool or are excreted into the bile. Biliary folate is then reabsorbed and distributed to the liver and other tissues.

Biliary folate, 5-methyl THF, is the same form as plasma folate. Plasma folate concentration has been found to be much more dependent on biliary folate than on dietary folate (Baker et al, 1965; Steinberg et al, 1979; Colman, 1981). Pratt and Cooper (1971) found bile in humans fed a labeled dose of folic acid or 5-formyltetrahydrofolic acid contained a similar variety of folates including a rapid influx of the label. This suggested that bile folates represent those present in the liver pool rather than simply excretion of newly absorbed folate arriving at the liver via the portal vein.

There is a delay before folate concentration increases in systemic and hepatic-vein blood after an oral dose of folate. Then large amounts of folic acid and methylfolate appear in the blood leaving the liver. This indicates that liver is the major site of folic acid metabolism (Whitehead and Cooper, 1967). The major portion of liver folates are pteroylpolyglutamates as demonstrated by increased response of human liver tissue to L. casei after the liver extract was mixed with human serum which contains conjugases that hydrolyze long chain polyglutamates (Whitehead, 1973). In

the liver more than 85 percent of folate was found to be polyglutamates containing more than three glutamyl residues.

Plasma Folate

Circulating folate in plasma is the monoglutamate form 5-methyltetrahydrofolate and is completely assayable by L. casei activity (Hoffbrand et al, 1977). Three factors have been identified as determining the magnitude of serum or plasma folate rise after an oral folate dose in a folate preloaded state. One is the extent and rate of absorption of folate derivatives from the intestinal mucosa into the portal blood. The second is exchange with liver folate and perhaps other tissue folate stores. The third is the rate of urinary excretion of circulating folates (Brown et al, 1973).

Red Blood Cell Folates

Detection of the polyglutamate forms of folate by growth of L. casei requires prior deconjugation with a conjugase. The response of whole blood extracts to L. casei increases more than ten-fold following treatment with chicken liver conjugase indicating that most folate in blood is polyglutamate (Noronha and Aboobaker, 1963). Methylated forms of folate do not support growth of S. faecalis or P. cerevisiae. Red blood cell folate supports only ten percent as much growth by S. faecalis and P. cerevisiae as L. casei indicating that the preponderance of folate present is

methylated (Shin et al, 1974). Red blood cells contain mostly pentaglutamates as well as some tetra- and hexaglutamates (Perry et al, 1976). In B_{12} deficiency and in primary folate deficiency, there is no change in the type of polyglutamates present in red blood cells. But in B_{12} deficiency there is a decrease in polyglytamate concentration, while in early folate deficiency short chain folates are decreased. This means that in B_{12} deficiency, polyglutamate synthesis is impaired due either to lack of substrate or a specific B_{12} requirement for synthesis.

Active Coenzyme Folate Form

Cellular folate, the active coenzyme form, is formed from tetrahydrofolate. Vitamin B_{12} is necessary for the homocysteine-methionine reaction which converts methyl tetrahydrofolate, the circulating form taken up by cells, to tetrahydrofolate, the active form. This phenomenon is sometimes referred to as the "methyl trap". In a B_{12} deficiency state, the reaction does not proceed adequately and methyl tetrahydrofolate accumulates in plasma leading to insufficient tetrahydrofolate which is the active coenzyme form of folate (Stokstad et al, 1981). Decreased polyglutamate synthesis causes decreased capacity to retain intracellular folate.

Folate coenzymes participate in several enzymatic reactions. These reactions are necessary for purine and pyrimidine synthesis, formation of methionine from

homocysteine, the catabolism of other amino acids including histidine, interconversion of serine and glycine and oxidation-reduction reactions of some folic acid derivatives (Rosenberg and Godwin, 1971).

Folate polyglutamate synthetase is the enzyme present in cells which catalyzes the formation of active polygutamates from monoglutamates (Anonymous, 1983).

Dietary Folate

Individual Intake vs RDA

The term folic acid was introduced by Mitchell in the 1940's (Mitchell et al, 1944) to describe a factor present in leafy vegetables which had the same growth-supporting properties for microorganisms as the liver L. casei factor. Although folate is present in most natural foods, the greatest amounts per unit of dry weight are in yeast, liver and other organ meats, fresh green vegetables and some fresh fruits. Herbert (1981) suggested that daily consumption of one fresh or fresh-frozen uncooked vegetable, fruit or fruit juice could prevent folate deficiency. Uncooked food sources are emphasized because folates are susceptible to oxidative destruction. As much as 50 to 90 percent of this vitamin is lost during refining and cooking. Hoppner et al (1972) found fresh vegetables contained almost ten times as much total folate activity (5.6 to 203.5 mcg/100 g) as fresh frozen vegetables (9.8 to 32.9 mcg/100 g) in the Canadian food supply. Although milk is not considered a rich source

of folate, folate present in fresh milk is absorbed to a greater extent than folate from other foods due to the presence of a facilitating factor (Colman,et al 1981).

The Recommended Dietary Allowance (RDA) for adults for folate in 1968 was established at 400 mcg total folate per day (National Research Council, 1968). The 1980 RDA for folate is 400 mcg for adults and for children over 10 years old, 800 mcg during pregnancy and 500 mcg during lactation (National Research Council, 1980). Folacin allowances are based on dietary sources as assayed by L. casei after conjugase treatment to make polyglutamyl forms of the vitamin available to this test organism. In contrast to the 400 to 800 mcg RDA, the minimum human requirement for folate is believed to be about 50 mcg daily (Herbert, 1962a). The eightfold difference between the RDA for folate and the true requirement is the result of incomplete knowledge about the utilization of naturally occurring folates plus a generous safety margin to allow for folate losses during cooking, storage and processing (Dong and Oace, 1973).

Estimates of daily folate inake calculated from dietary records and published food folate values consistently fall far below the RDA. Yet the incidence of sub-normal folate stores in the population is not correspondingly high. Bates et al (1982) proposed that this discrepancy is the result both of an underestimate of food folate intake and an overestimate of the need for folate. Underestimates of food folate content can result from destruction of food folate

prior to its assay, differing L. casei growth response for various forms of biologically active folate monoglutamates, the use of different deconjugation enzymes, and presence of conjugase inhibitors in foods.

Recommendations to lower the RDA from a level viewed as unnecessarily high have been advanced by Herbert (1977) and Cooper (1978) with decreases to 150 mcg and 300 mcg/day for adults recommended, respectively. Cooper's recommendation is based on Canadian data. Only eight percent of men and 10 percent of women had low erythrocyte folate levels while food survey information indicated that the mean folate intake was only 192 mcg/day for men and 147 mcg/day for women. Bates et al (1982) suggested that the RDA is overestimated due to its basis on experimentally induced deficiency. These requirements may not match those of a normal population. This causes an overestimate in the amount needed to "maintain" normality. Further, the safety factor built into the recommendation is exceptionally large to include variations in availability from food and in absorption.

Food Folate Forms and Their Availability

The earliest comprehensive compilation of the analyzed folate content of foods was USDA Handbook #29 (Toepfer et al, 1951). These values were demonstrated to be unrealistically low. Analyses were performed without the addition of ascorbic acid to samples to prevent the

oxidation of labile forms of folate during the assay (Chen et al, 1983). Hurdle and others (1968) demonstrated that addition of ascorbic acid during extraction and assay of hospital foods, even without the addition of conjugase, increased folate values to 1.2 to 40 times those of Toepfer's analysis in which conjugase but no ascorbic acid was used. This discrepancy was greatest in vegetables implying that concentration of heat-labile free folate was greater than conjugated folate in vegetables.

Perry (1971) analyzed cooked western meals containing meats, vegetables, potatoes, fruits and milk for total and free folate content using ascorbate and conjugase. The mean free folate content of the meals was 53 mcg whereas the mean total folate was 497 mcg. The release of folate activity for the assay microorganism in food analyses or for absorption by animals is accomplished by hydrolysis of additional glutamates by a proteolytic enzyme, a conjugase (Hurdle et al, 1968). Conjugase incubation of cooked and uncooked foods in a hospital diet produced 1.24- to 32-fold increases in assayed folate values over non-incubated samples.

Vegetable and animal tissues contain endogenous conjugases which free pteroylmono- and diglutamates from polyglutamates and make them available for absorption (Reed et al, 1976). Leichter et al (1979) demonstrated that endogenous conjugase was present in fresh vegetables when they found that free folate activity was significantly

higher in vegetables boiled after homogenization than in those boiled before homgenization. The implication is that the increase in free folate was variable due to the positive effects of endogenous conjugases on polyglutamates and the negative effect of inactivation of conjugase upon boiling. Total folate was not significantly different between the two methods.

When ingested by itself, 90 percent of folate monoglutamate is absorbed by humans. This amount may be decreased in the presence of other factors in food (Herbert, 1981). Polyglutamates must be hydrolyzed to mono- and diglutamates for absorption. Conjugase activity in the human intestine is plentiful relative to the amount of polyglutamate to be hydrolyzed. Decreased availability of polyglutamate can occur; this is due to a decrease in the activity of conjugase, due to presence of a conjugase inhibitor, not a decrease in the total amount of conjugase present. Stokstad et al (1977) found folate conjugates from orange juice concentrate to be poorly absorbed due to inhibition of conjugase activity by a low pH. Conjugase inhibition also has been demonstrated during food folate assays by Santini and others (1962). Total assayed folate value of Puerto Rican foods was found to be much higher after addition of chicken pancreas extract, a source of conjugase, when incubation occurred after rather than before filtering. This implied that the conjugase inhibitor was eliminated during the filtering process.

Dong and Oace (1973) cautioned that assayed food folate values alone should not be accepted as amounts available to humans. The efficiencies of the specific metabolic reactions involved in the absorption and transport of folate are not completely understood. Some chemical folate forms may be preferentially used under specific physiological conditions and certain drugs and components of food may inhibit enzymes involved in folate metabolism.

Folate Content of Processed Foods

Folate is labile to oxidation during heating. Since a large portion of food consumed in the U.S. is heat processed, folate availability has been a subject of interest. Hurdle (1968) studied the effect of cooking on folate values in a hospital diet. Although meats lost relatively little folate activity during cooking, vegetables lost up to 90 percent, oatmeal lost 80 percent and egg yolk lost 30-70 percent during cooking. Hoppner (1971) measured folate availability in canned, strained baby foods and found that canned, strained fruits and vegetables had much lower total folate activity than similar fresh foods. Vegetables ranged from 9.9 mcg/100 g for beets down to 2.5 mcg/ 100 g for carrots whereas fruits ranged from 2.1 mcg/100 g for bananas down to .6 mcg/100 g for apricots. Meats lost much less folate in processing. Hoppner et al (1973) also determined the folate activity in 30 frozen convenience dinners. They found that the dinners which included a meat,

a vegetable and a potato or cereal had higher folate content than other types of meals. Average folate content was 12 mcg/100 kcal or 41 mcg/dinner. Total folate was not reduced by reheating after defrosting.

The folate content of individual foods must be considered separately rather than in categories of similar foods such as fruits or vegetables because foods within each category have large differences in folate content. Specific food choices within categories of Women, Infants and Children (WIC) program foods were analyzed for total folate value by Thenen (1982). The total folate content of the high folate choices in all categories was 554 mcg/day whereas the low folate choices provided only 50 mcg/day.

Folate Intake Data

Folate intake among groups of people has been calculated most frequently based on national food disappearance data, household food consumption surveys and individual dietary records. Hoppner et al (1972) examined the free and total folate content of the average Canadian diet based on food disappearance data. Calculated folate intake was 140 mcg/day of free folate and 240 mcg/day of total folate per person. Consumption of categories of foods was estimated and the total folate value for each group was estimated for Canadian households. Meat, fish and poultry contributed the largest amount of folate, 59 mcg/day, with cereal products second at 36 mcg/day, dairy products at 33

mcg/day and potatoes at 21 mcg/day. Leafy vegetables and legumes, considered two of the most concentrated sources of folate, together accounted for only 20 mcg/day. This skewed intake distribution is caused by differences in the relative quantity of foods in each group which were eaten (Hoppner et al, 1977). Daily per capita consumption of dairy products was 493 g. Daily intakes of meat products (194 g), cereals (260 g), and potatoes (178 g) were high relative to the amounts of leafy vegetables (38 g/day) and legumes (31 g/day). Bates and others (1982) calculated that the mean daily total folate intake in the UK was 190 mcg based on the 1976 National Food Survey data. Spring et al (1979) calculated the folate intake attributed to food groups from the 1976 British survey. Fresh and processed vegetables made the greatest daily contribution (54 mcg) followed by bread, (38 mcg) and liquid milk (20 mcg). Meat and fish provided only a low amount (8 mcg). This distribution is closer to that expected based upon the folate content of foods in each category and differs markedly from the Canadian distribution (Hoppner et al, 1972).

Household folate consumption in the UK also was calculated based on the 1979-1981 National Food Survey (Poh Tan et al, 1984) and was estimated to be 210-213 mcg/person/day. The increase from the previous survey was attributed to including the folate provided by tea which totaled 16 mcg folate/person/day. In this study, food groups also were analyzed and the distribution of folate

intake again differed from that of the Canadian study. Bread was the greatest contributor to dietary folate (18 percent of total), fresh green vegetables were next (12 percent) and frozen and processed vegetables were third (9 percent). Meats as a group contributed the least to folate intake (7 percent of total).

Folate in cooked Puerto Rican diets was analyzed microbiologically by Santini and Corcino (1974). Total folate content was 1314 mcg/day for a low cost diet and 2345 mcg/day for a high cost diet. These comparatively high intakes of folate are attributable to high intakes of fruits, vegetables, legumes and grain products.

Folate intake among various age groups in the United States has been investigated. Breskin and others (1985) calculated folate intake of children 40-108 months old from 2-day dietary records. For reference, adequacy was set at 8-10 mcg folate/kg body wt/day. Children who received no supplement had a mean folate intake of 9.1 mcg/kg/day while supplement users consumed 20-24 mcg/kg/day from food plus supplements. Variation among individuals was large. Several children had folate intakes below 7 mcg/kg/day. Riester and Waslien (1975) reported folate intake of nine year old girls, calculated from dietary records, was 74.9 mcg/day. This is less than 25 percent of the RDA.

Among a sample of pregnant girls under 16 years old, Daniel et al (1971) found that 90 percent had folate intakes of less than 50 percent of the RDA and 52 percent had

intakes below 10 percent of the RDA. Other studies of teen-aged girls have calculated average folate intakes. Based on 3-day dietary records, intakes were 25 percent and 16 percent of the RDA (Van de Mark and Wright, 1972) among pregnant and non-pregnant girls, respectively. Intakes were 243 mcg/day for white and 185 mcg/day for black teen-aged girls (Liebman, 1985); and 210 mcg/day among urban and 191 mcg/day among rural teen-aged girls (McCoy et al, 1984). McCoy found a wide range of folate intakes between individuals based on two daily dietary recalls. Of all nutrients studied, folate was the nutrient whose intake was most frequently below 67 percent of the RDA. The mean daily folate intake of Arkansas girls, based on two one-day dietary recalls, was 204 mcg for 12 year olds, 216 mcg for 14 year olds and 203 mcg for 16 year olds (Dutram, 1984). The mean folate intake of Oklahoma girls of the same age range was 215 mcg/day (Lan, 1982).

Adult women have higher folate intakes than teen aged girls. Eighteen women's folate intakes averaged 230 mcg/day based on dietary records over a period of three months (K.Yadrick, unpublished data). Diet records of 151 middle-aged women on two randomly-selected days per month for one year showed mean consumption of 252 mcg folate per day (Sempos et al, 1984). Still, 65 percent of the women had mean daily intakes below 70 percent of the RDA and 23 percent were less than 50 percent of the RDA.

Calculated and analyzed folate content of food intake have been compared. Total folate content was calculated and analyzed microbiologically for typical high cost, low cost and "poor" American diets (Chung et al, 1961). Analyzed daily total folate intake was approximately one-third higher than calculated folate intake. The analyzed folate values were 93 mcg for the high cost diet, 157 mcg for the low cost diet and 47 mcg for the poor diet. Both the calculated and analyzed values were erroneously low because S. faecalis was used for microbiological assays. This organism only uses non-methyl folate forms (Dong and Oace, 1973). Yet the discrepancy between calculated and analyzed values is noteworthy. More recently, Moscovitch and Cooper (1973) compared the folate content of 7 and 4 day dietary intakes as 1) calculated from dietary recalls, 2) assayed from duplicate samples prepared by subjects, and 3) assayed from hospital-prepared duplicate samples. The assayed hospital-prepared foods had higher folate content (383 mcg/day for 7-day records) than home-prepared samples (242 mcg folate/day). Calculated folate was only 105 mcg/day. These results imply that calculated folate values are only about 25 percent of measured values and that storage and preparation methods can decrease the folate content of foods. Table 1 shows a summary of reported folate intake data.

Table 1. Reported data on mean daily total folate intake.

Source	Age Group	Method	Mean Daily Total Folate Intake	RDA
			Mcg	Mcg
Hoppner et al (1972)	All of Canada	Food Disappearance	240	400
Van de Mark and Wright (1972)	Pregnant Girls Non-Pregnant Girls	3-Day Dietary Record	128 100	800 400
Santini and Corcino (1974)	All of Puerto Rico	Food Analysis	1314-2345	400
Riester and Waslien (1975)	Nine Year-Old Girls	Dietary Record	75	400
Bates et al (1982)	All of UK	Food Disappearance	190	400
Lan (1982)	Teenage Oklahoma Girls	2-Day Dietary Record	215	400
Dutram (1984)	12 Year-Old Girls 14 Year-Old Girls 16 Year-Old Girls	2-Day Dietary Record	204 216 203	400 400 400
McCoy et al (1984)	Urban Teenage Girls Rural Teenage Girls	2-Day Dietary Record	210 191	400 400
Poh Tan et al (1984)	All of UK	Food Disappearance	210	400
Breskin et al (1985)	3 to 9 Year-Olds	2-Day Dietary Record	9.1 mcg/kg	8-10 mcg/kg
Liebman (1985)	Black Teenage Girls White Teenage Girls	2-Day Dietary Record	185 243	400 400

Folate Status

Folate and Nutritional Anemia

Nutritional anemia is a condition in which the hemoglobin concentration is sub-normal due to a deficiency of one or more of the hemopoietic nutrients i.e. iron, folic acid and vitamin B_{12} (WHO, 1972 Baker and DeMaeyer, 1979). The low hemoglobin concentration of nutritional anemia can be raised by increasing the amount of the deficient hemopoietic nutrient. Anemia is an overt symptom of deficiency and is preceded by measurable biochemical and/or chemical abnormalities. Anemia, per se, generally is not associated with increased morbidity or impairment of body function, but anemia does reduce the capacity for oxygen transport.

With folic acid deficiency, the following biological changes occur sequentially. First, serum folate (SF) decreases and hypersegmentation of neutrophils is evident. Next, urinary formiminoglutamic acid (FIGLU) increases. Finally, red blood cell folate (RBCF) decreases, macro-ovalocytosis occurs, and megaloblastic marrow changes and anemia are detectable (Herbert, 1962a).

Bills and Spatz (1977) found neutrophilic hypersegmentation was a good early indicator of incipient folate deficiency. Among a group of subjects with neutrophilic hypersegmentation but who had no other signs of anemia and a control group without hypersegmentation a

significant negative correlation was detected between the number of neutrophil lobes and SF level. Hages and Pietrzik (1985) found an increase in the average number of lobes of neutrophilic granulocytes in children 1-15 years old was associated with SF concentrations below 4 ng/ml or RBCF concentrations below 250 ng/ml. Low circulating folate levels in serum and red blood cells are not regarded as a basis for treatment in the absence of other signs of folate deficiency anemia such as glossitis and hypersegmentation of skin and mucosa, megaloblastic changes in developing red blood cells, and impairment of DNA synthesis as manifested by morphological changes in hemopoietic cells and other dividing cells in the body (Izak et al, 1963). Low circulating folate levels, however, have been used in nutritional surveys to estimate the prevalence of depleted folate stores in populations. SF is thought to reflect recent folate balance. SF concentration correlates poorly with hepatic, bone marrow, red cell and whole blood values in non-anemic people (Rodriguez, 1978).

RBCF is less sensitive to short-term variation in folate balance and reflects body folate stores. As much as 10 percent of apparently healthy individuals may have low blood cell folate values though many people with low RBCF levels have normal hematological status. The incidence of folate deficiency as detected by RBCF concentration among a sample of non-anemic pregnant African women was 43.8 percent

(Colman et al, 1975). Hemoglobin and red cell volume were normal among women despite a RBCF deficiency.

Low red blood cell levels of folate may represent depletion of tissue stores and an increased risk of megaloblastosis. Virtually all people with megaloblastic anemia have below-normal SF and RBCF (Senti and Pelch, 1985). Erythrocyte folate concentration was compared between 57 normal subjects, 20 iron deficient (Hb 3.8-10.9 gm/100 ml), and 23 folate deficient (plasma folate <2 ng/ml and serum B_{12} > 150 pg/ml) (Omer et al, 1970). Mean erythrocyte folate concentrations were 183 ng/ml for controls, 336 ng/ml in iron deficient and 51 ng/ml in folate deficient subjects. Treatment with iron decreased erythrocyte folate levels to normal in the iron deficient subjects. A failure of folate utilization in early red cell development may explain the high RBCF concentration during iron deficiency.

Population Surveys of Folate Status - Large Studies

The Ten-State Nutrition Survey of 1968-1970 was the first large scale survey of the folate status of the U.S. population (Ten State Nutriton Survey, 1972). Only average SF and RBCF concentrations for each age group studied were presented. Mean SF values by age group were; 13-16 years : 5.87 ng/ml, 17-44 years : 6.2 ng/ml and 45-59 years : 6.95 ng/ml and RBCF values; 13-16 years : 199 ng/ml, 17-44 years : 236 ng/ml and 45-59 years : 249 ng/ml. All

were within the normal range although those for the youngest age group were lowest. Mean blood folate values can obscure large numbers of abnormally low values because variation among individuals is great. A report of the Massachusetts data from the Ten State Study (J.C. Edozien, 1972, unpublished) showed that in low-income districts surveyed, 26.1 percent of all RBCF were below 160 ng/ml. But the 60 years and older population had the highest incidence of low RBCF levels; it was 30.7 percent among females and 28.4 percent among males.

The Nutrition Canada National Survey of 1970-1972 (Nutrition Canada, 1973) assessed folate status by measuring SF only. Ten percent of children and adolescents were assessed to be in the high risk category (SF below 2.5 ng/ml) in folate status while almost 25 percent of adults were at high risk. The prevalence of high risk was greatest among Eskimos in both age groups. Nutrition Canada found that young adult women (20-39 years old) had relatively poor folate status [(21.2 percent high risk (<2.5 ng/ml) and 46.7 percent moderate risk (2.5-5 ng/ml)]. There is little data on the folate status of young adult women although it might be expected that pregnancies and parity would be expected to lower folate stores in this group.

The Second National Health and Nutrition Examination Survey (HANES II) of 1976-1981 did not collect serum and red blood cell folate data to assess folate nutritional status of the U.S. population (McDowell et al, 1981). Folate data

was collected only as an aid in interpreting other data on subjects with abnormal hematological indices. Reference data were also collected from a random sample of ten percent of the HANES II study population. The highest percentage (13 percent) of low RBCF values was among 20-44 year old females. Low SF and RBCF concentrations were not accompanied by symptoms of anemia, low hemoglobin, increased mean corpuscular volume or large numbers of macrocytic red blood cells. There was no difference between blood folate levels attributable to sampling time i.e. fasting vs nonfasting (Senti and Pilch, 1985).

Surveys of Folate Status Across Age Groups

Several small scale surveys of folate status across age groups have been conducted. Prothro et al (1976) assessed the nutritional status of 41 families (102 individuals) in Alabama and found that the most extensive nutrient deficiency based on serum indices was folate. Mean SF for each age group studied was between 3.8 and 4.8 ng/ml which is low but not deficient. Of the 102 individuals, three preschoolers, four adolescents and 14 adults had deficient serum levels (<3 ng/ml). Folate status was measured among 562 Seventh Day Adventists, 431 of whom were vegetarians (Armstrong et al, 1974). Vegetarians had significanly higher mean SF than non-vegetarians. Low B_{12} status was positively correlated with low RBCF values. A significant

inverse relationship between SF and serum B_{12} was detected as might be expected.

Folate Status in Pregnany

As folate is used for protein synthesis, requirements increase during periods of growth. The World Health Organization (WHO, 1972) has identified women in late pregnancy as a group at risk for low folate status. Collaborative WHO studies of women in their third trimester of pregnancy in less developed countries demonstrated deficient SF levels (<3 ng/ml) in 1.4 to 46 percent of subjects and borderline levels (3-6 ng/ml) in 16 to 92 percent of women studied. Low hemoglobin levels were correlated with low serum iron and low SF but were not associated with B_{12} level. Bailey et al (1980) found that folacin deficiency was more common than iron deficiency among a group of low-income pregnant women. Forty percent of the women had hematocrits below normal. Serum iron was normal in 96 percent and transferrin saturation was normal in 88 percent of the women, but RBCF levels indicated high risk (<140 ng/ml) in 29 percent and SF indicated high risk (<2 ng/ml) in 15 percent of the women. Herbert and others (1975) found that the mean SF and RBCF of low income pregnant women was in the normal range, yet 30 percent had RBCF levels suggestive of deficiency (<200 ng/ml) and 16 percent were overtly deficient (<150 ng/ml). Twenty percent had deficient serum levels (<3ng/ml). This study

illustrates how a wide variation in blood folate levels among individuals can be obscured by the mean value and can cause deficiencies to be overlooked.

The variation of folate stores during pregnancy was examined among Norwegian neonates and mothers (Ek, 1980). The plasma folate of the infants was 6 to 8 times that of mothers and infant RBCF was two times that of mothers. Term infants had significantly higher RBCF than those of lower gestational age. The data indicate that folate is transferred from the mother to the fetus during the last weeks of pregnancy. Correlations between folate status for pregnancy outcome have not been detected. Daniel et al (1971) found that 17 percent of pregnant girls under 16 years old had blood folate levels below normal and that this group had a similar incidence of premature delivery as did the non-deficient girls.

Folate Status in Adolescence

Adolescents are another population group considered to be at risk for folate status due to their rapid growth and maturation. The Ten-State Nutrition Survey of 1968-1970 (1972) reported that women 13-16 years old had a mean RBCF value (199 ng/ml) which was considered to be borderline. This was lower than older women(17-44 years; mean = 236 ng/ml and 45-59 years; mean = 249 ng/ml). The potential for mean values to mask large numbers of deficient individuals

encouraged study of folate status among adolescents in the following years.

Daniel et al, (1975) found plasma folate concentration decreased with increasing maturity among healthy adolescent boys and girls. Plasma folate was subnormal in 9.4 percent of all boys studied and in 4.7 percent of the low income girls. Mean plasma folate was below 6 ng/ml for all groups studied. Bailey et al (1982a) also found SF was lower with increasing age and sexual maturity among low income black and white adolescent males and females. SF was less than 6 ng/ml in 68 percent of the blacks and in 56 percent of the whites in the sample. RBCF was below 140 ng/ml in 63 percent of the blacks and 45 percent of the whites. SF and RBCF were correlated positively .

Similar values were found among 192 low-income, urban black and Spanish-American adolescents. RBCF below 140 ng/ml was found in 42 percent and SF was less than 6 ng/ml in 45 percent of the individuals (Bailey et al, 1982b). These data suggest that tissue folate stores are depleted during the adolescent growth spurt. Liebman (1985) found RBCF levels were below 140 ng/ml in 32 percent of the adolescent girls measured. Black girls consumed significantly less folate (185 mcg/day vs 243 mcg/day) and were more likely to have low RBCF concentrations (40 percent vs 27 percent) than white girls. Clark and Gates (1983) also found folacin status of 103 black and white Alabama adolescent girls to be very low and the percent who were

deficient increased with advancing age. Among 12, 14 and 16 year old girls, the percents with deficient SF were 7.7, 12.2 and 17.4, respectively. The corresponding percentages with deficient RBCF concentrations were 38.5, 46.3, and 65.2 percent. Ercanli et al (1984) found black low-income adolescent girls had a mean plasma folate concentration of 4.12 ng/ml. A corresponding high income group had an even lower mean, 3.77 ng/ml. SF was also measured among 144 Oklahoma adolescent girls. Sixty-three percent had normal SF, 19 percent had low levels (3-6 ng/ml) and 18 percent had deficient levels (<3 ng/ml) (Lan, 1982).

Folate Status Among Young Children

One study of 40 to 108 month old children found SF and RBCF were higher than they were in older age groups. Mean SF was 26.6 ng/ml and mean RBCF was 454.6 ng/ml among children not taking folate-containing supplements whereas the mean SF was 36.4 ng/ml and mean RBCF 622.6 ng/ml among children taking supplements containing folate (Breskin et al, 1985). Folate intake and RBCF, but not SF, were significantly positively correlated among the non-supplemented children.

Folate Status Among the Elderly

Webster and Leeming (1979) compared RBCF levels of 25 young controls (mean age 27 years) with those of three groups of elderly people: 75+ years old living at home; 75+

years old hospitalized with acute illness; and 65+ years old with subnormal (<100 ng/ml) RBCF levels. Among the elderly, those living at home had the highest incidence of low RBCF (24 percent) while the acute and long-term ill hospitalized groups had 16 and 18 percent, respectively, with low RBCF levels. These results may reflect folate supplementation in a hospital or inadequate folacin intake among elderly living independently.

Among a group of low income elderly in Miami, Florida, 60 percent had RBCF of <140 ng/ml while their B_{12} and iron parameters were normal (Wagner et al, 1981). One explanation for the high incidence of low blood folate was the consumption of fresh vegetables by only 17 percent of the sample and citrus daily by only 30 percent. Extensive boiling of vegetables also was reported to be a common cooking method. High income elderly women in the same geographic area had only a 6 percent incidence of low RBCF. Income probably influenced the dietary habits of these two groups of people.

Among an elderly population in Wales, mean plasma folate was normal in both males (7.0 ng/ml) and females (6.7 ng/ml). The number with plasma folate below normal (<5 ng/ml) was 62 of 229 males and 99 of 304 females. Mean RBCF was 341.1 ng/ml among males and 317.7 ng/ml among females. Nineteen of 229 males and 24 of 229 females were below normal (<200 ng/ml) (Elwood et al, 1971).

Hayes et al (1985) found SF levels to be low in only three percent of a healthy reference group and 7.7 percent of a hospitalized group of elderly. RBCF was low in only 1.5 percent of the healthy group and 9 percent of the hospitalized group. Folate status of the elderly has been summarized by Rosenberg et al (1982) as follows: folate deficiency does not appear to be widespread among the free-living elderly. Poor, hospitalized and institutionalized elderly may be at greater risk amd alcoholism may also contribute to low folate status among the elderly.

The Human Folate Requirement

In 1962 Herbert demonstrated the response to induced folate deficiency in one healthy male (Herbert, 1962a). On a diet providing only 5 mcg total folate daily, SF dropped to 3 ng/ml in three weeks. By seven weeks, the average number of lobes of the nuclei of polymorphonuclear leukocytes had increased. By fourteen weeks, urinary FIGLU excretion increased due to blocked histidine catabolism. By eighteen weeks, macrocytic anemia was evident. Liver folate stores were estimated at 3.5 - 7.5 mg. Since it took 4.5 months for a deficiency to appear, Herbert calculated that the adult folate requirement was approximately 50 mcg/day.

A limited number of short term trials of folate depletion and supplementation have been conducted in an attempt to clarify the maintenance requirement for folate in healthy people. Herbert (1962b) measured the amount of

pteroylglutamic acid (PGA) required to maintain normal SF in three healthy adult females consuming a diet containing 5 mcg/day of total folate activity. The three subjects received supplements of 25 mcg, 50 mcg or 100 mcg PGA daily in addition to a multivitamin capsule without folate. The SF of the subject who received only 25 mcg supplemental PGA daily fell below normal in four weeks and was below 5 ng/ml within six weeks. With the 50 and 100 mcg supplements, SF remained above 6 ng/ml for the entire six weeks. These data support the contention that 55 mcg folate/day is adequate for maintenance of adults.

Banerjee et al (1975) determined the folate requirement for normal Indians to be approximately 75 mcg/day. Six adults were fed a diet containing 15 mcg total folate per day with supplements of 25 mcg, 50 mcg, 75 mcg or 100 mcg PGA daily. Normal RBCF was achieved and/or maintained only at intakes of 90 mcg or greater. SF was maintained at normal levels only with intakes of 115 mcg/day PGA.

The folate requirement for reversal of megaloblastic anemia also has been investigated. One subject with anticonvulsant-associated megaloblastic anemia (Druskin et al, 1962) responded positively to 25 mcg/day folate and after 41 days her hemoglobin was 13.4 g/100 ml blood. These results indicate that less than 50 mcg supplemental folate/day would reverse folate depletion. In contrast, pregnant patients with megaloblastic anemia eating a diet providing 45 mcg/day free folate were found to need 500 to

1000 mcg PGA daily to elicit a positive hematological response. After delivery, however, anemia could be corrected by a folate supplement of as little as 50 mcg/day, the same amount required by normal non-pregnant women (Pritchard et al, 1969).

Short Term Experimental Low and High Folate Diets

A few studies have investigated short term responses in SF and RBCF to changes in folate intake. After 30 and 35 days on a very low folate diet (17-34 mcg total folate), SF fell in two normal subjects but erythropoiesis was maintained at 3-4 times its normal rate during phlebotomy (Eichner et al, 1971). Two alcoholic subjects with megaloblastic anemia were fed a high folate diet (150-200 mcg/day free folate) for 19 and 11 days, respectively (Eichner et al, 1971). SF increased from less than 4 ng/ml to almost 6 ng/ml in 11 days for one subject and from less than 2 ng/ml to almost 7 ng/ml in 19 days for the other subject. On a subsequent low folate diet (less than 20-25 mcg/day free folate), the SF of both subjects dropped to approximately 3 ng/ml. The serum of both continued to drop on the low diet until it leveled off at about 1.5 ng/ml after 45 days.

PGA supplements of 400 mcg/day for 60 days increased mean SF by 4.8 ng/ml and RBCF by 91 ng/ml among twenty four 11 to 15 year old adolescents (Tsui et al, 1985). Over half of the experimental group had SF increases of 100 ng/ml or

more indicating tissue stores were less than saturated. Increases in hemoglobin (0.4 g/dl) and decreases in mean corpuscular volume (1.8 u^3) with the supplement suggest that some subjects were folate deficient.

Campillo et al, (1985) supplemented a group of hospital patients consuming a diet providing approximately 300 mcg folate/day, 50 percent of whom were folate deficient. Among the group receiving 5 mg/day PGA supplement for seven days, mean SF was increased on day 8 from 5.8 to 14 ng/ml, and mean RBCF had increased from 265 to 320 ng/ml. Among the group receiving a 50 mg PGA supplement for only one day, mean SF had increased on day 8 from 5.2 to 8.2 ng/ml and mean RBCF had increased from 194 to 269 ng/ml.

Eichner and Hillman (1973) placed eight subjects on a diet containing only 15 to 25 mcg/day total folate. SF decreased by 9 percent after one day and 30 percent by day six of the diet in subjects who had been supplemented with 150-250 mcg free folate/day for 4-10 days previously. Sixty women consumed a self-selected folate restricted diet for two weeks (mean calculated total folate intake of 159 mcg/day). Individuals were supplemented daily with 100 mcg of PGA, orange juice containing 100 mcg total folate, or received no supplement daily for the subsequent seven weeks on the restricted diet. During the two weeks on the folate-restricted diet without folate supplements, mean SF of subjects decreased to 62 percent of its initial value. SF was increased significantly with both supplements.

Increases with the two supplements did not differ sidnificantly. These data indicate that folate intake of 259 mcg/day (below the RDA of 400 mcg/day) increased SF in women. RBCF changes were not reported.

Effects of Oral Contraceptive Agents and Other Drugs on Folate Status

Oral contraceptive agents (OCA) may alter folate status of women. NHANES II (Senti and Pilch, 1984) reported that OCA use was associated with folate status of 20-44 year old females but the relationship was not statistically significant. A study of 526 medically indigent women attending a family planning clinic detected no significant difference between mean SF of OCA users and non-users (Paine et al, 1975). Mean hemoglobin, however, was higher among OCA users, presumably from reduced menstrual blood losses. Pietarinen et al (1977) found SF was significantly lower among OCA users on day five but not on day 20 of the menstrual cycle.

In their supplementation study, Rhode et al (1983) found initial serum and RBCF levels to be significantly lower in OCA users than in non-users. But on both the restricted and supplemented diet, SF values of both groups converged and significant differences between OCA users and non-users disappeared. These authors suggested that the lower initial SF of OCA users was due to lower intake of folate or interference of OCA with utilization of high-folate foods other than orange juice.

Folate status of 74 adolescent OCA users and 96 non-users was compared by Grace et al (1982). Among OCA users, SF was lower than 5 ng/ml in 37 percent and whole blood folate below 150 ng/ml in 33 percent, while only 21 percent and 15 percent of non-OCA users had low serum and whole blood folate levels, respectively.

Other drugs including certain antimicrobial agents (Shojania and Hornady, 1969), anticonvulsant drugs (Kelly et al, 1979), and aspirin (Shevchuk and Roe, 1985) have been shown to interfere with folate utilization and may negatively affect long term folate status. Alcohol also is known to impair hepatic folate metabolism (Lindenbaum, 1980; Hillman and Steinberg, 1982: and Russell et al, 1983).

CHAPTER III

CHANGES IN SERUM FOLATE CONCENTRATION IN RESPONSE TO SELF-SELECTED OR LOW FOLATE AND HIGH FOLATE DIETS

Abstract

A low folate (74 mcg total folate) and high folate (low folate plus 400 mcg PGA) diet were provided for one week periods to 12 female volunteers 22 to 41 years of age to determine short term responses in serum folate (SF) and red blood cell folate (RBCF) concentrations. In a preliminary week, subjects consumed a self-selected diet and kept detailed dietary records. Six of the subjects used oral contraceptives (OCA) while the other six did not (non-OCA). SF was measured on days 3, 5 and 7 of each week while RBCF and hematocrit were measured on day 7 of each week. RBCF was lower ($P<.05$) during the preliminary week and SF was lower ($P<.02$) during the treatment periods for OCA than non-OCA. SF was greater ($P<.01$) with the high than the low folate diet (11.8 vs 8.0 ng/ml) by day 7 of each period. RBCF was not markedly altered by folate intake (239 vs 228 ng/ml for the high and low folate diets, respectively). Rapid response of SF to folate intake implies that SF is useful as a measure of short term folate nutriture.

Introduction

Folate deficiency is detected by sub-normal tissue (SF or RBCF) concentrations of folate or subnormal function of folate-dependent enzyme systems (1). Based on limited data, SF changes rapidly in response to dietary folate and reflects recent intake of folate. RBCF, in contrast, reflects body folate stores and longer term folate status. SF in one adult male fell below 3 ng/ml (2) in three weeks on a low folate diet (5 mcg/day total folate) and in one adult female consuming 30 mcg/day (3) SF fell below 5 ng/ml in six weeks. Mean SF fell by nine percent in one day and by 30 percent in six days among six males consuming a diet providing 5 mcg/d total folate (4). Mean SF of 60 adult women decreased by 38 percent of initial levels in two weeks on a self-selected folate-restricted diet calculated to provide 159 mcg/day total folate (5). Subsesquent supplementation with 100 mcg PGA or 100 mcg folate in orange juice per day increased mean SF (5).

SF is the biochemical parameter most frequently employed as an index of folate status. In surveys of nutritional status, a single measurement of SF alone or together with a RBCF measurement has been used frequently as an indicator of the folate status of population groups (6,7,8,9). A diet history, food frequency, or one or more 24 hour dietary recalls are usually obtained when blood samples are drawn.

Nutrition surveys indicate that young adult women are the population group at greatest risk for folate status (7,9). This group is also the most likely to use oral contraceptive agents (OCA) which are associated with decreased SF concentrations (5,10). Data on short term variation in SF concentration in response to folate intake are limited. The objective of this trial was to measure response in SF and in RBCF to folate intake by young adult women.

Methods

Twelve healthy female volunteers, 22 to 41 years of age, consumed a self-selected diet excluding nutrient supplements for a preliminary week (week 1) and maintained detailed daily records of all food and beverages consumed (APPENDIX A). During the following week (week 2) all subjects consumed a low folate diet with or without 400 mcg of supplemental folate per day with breakfast.[1] The basal diet provided 74 mcg folate per day according to analysis of the complete diet[2] (APPENDIX B). During week 3, diets for the two groups of subjects were switched. Hence, the experimental design was a crossover and results were statistically analyzed by removing variance attributable to subject and to week.

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[1]Nature Made Nutritional Products, Los Angeles, CA

[2]Hazelton Laboratories, Madison, WI

Subjects were living at home during the experiment and were trained to estimate their food intake using food models and measuring utensils. Dietary intake of folate and other nutrients was calculated for week 1 using a microcomputer program (11). All food for the experimental diet (Figure 1) for weeks 2 and 3 was provided to subjects but was consumed at home. Each food item was individually packaged and labeled by the day the diet was to be consumed. Subjects completed a daily check sheet documenting consumption of the test foods (APPENDIX A). The diet consisted of a two-day repeating menu. The low folate experimental diet was compiled from foods purchased in large quantities from single lots to minimize variation due to origin or processing of food items.

Six of the twelve subjects used oral contraceptive agents (OCA) while six did not (non-OCA). Subjects were stratified by initial folate levels within OCA group for assignment of diet order.

Prior to the start of the study, each subject completed a consent form and questionnaire about height, weight, supplement usage, menstrual cycle, alcohol, tobacco and drug use and was given a set of instructions for experimental procedures (APPENDIX C). The experiment was approved for use of human subjects by the Oklahoma State University Institutional Review Board.

Fasting SF was measured on days three, five and seven of each week. RBCF and hematocrit were measured on day

14 DAY EXPERIMENTAL DIET
(TWO-DAY ALTERNATING MENU)

4 OZ GRAPE JUICE	6 OZ APPLE JUICE
1 OZ CHEERIOS	1 OZ 100% NATURAL CEREAL
4 OZ MILK (2% OR SKIM)	4 OZ MILK (2% OR SKIM)
1 SLICE RYE BREAD	1 SLICE RYE BREAD
1 OZ AMERICAN CHEESE	1 OZ SWISS CHEESE
2 OZ TURKEY HAM	1 OZ BOILED HAM
3/4 CUP APPLESAUCE	1/2 CUP PINEAPPLE CHUNKS IN JUICE
2 FIG BARS	1 OZ HERSHEY BAR
4 OZ SIRLOIN STEAK	3 OZ CHECKEN BREAST PATTY
10 SALTINES	10 SALTINES
1/2 CUP PEACHES IN LIGHT SYRUP	3/4 CUP PLUMS IN HEAVY SYRUP
1/2 CUP VANILLA ICE CREAM	1 POPSICLE
1 NATUREMADE THERAPEUTIC M MULTIVITAMIN/MINERAL	1 NATUREMADE THERAPEUTIC M MULTIVITAMIN/MINERAL

MISCELLANEOUS OPTIONAL ITEMS AS DESIRED:
MARGARINE PATS, JELLY PACKETS, MAYONNAISE, CATSUP AND MUSTARD PACKETS, HALF & HALF PACKETS, LIFE SAVERS, METAMUCIL PACKETS, COFFEE, TEA, WHITE SUGAR, COKE/PEPSI, DR. PEPPER, 7-UP, HARD CANDIES.

CALCULATED TOTAL FOLATE PER DAY	43 MCG TOTAL FOLATE
ANALYZED TOTAL FOLATE PER DAY	74 MCG TOTAL FOLATE

PROXIMATE ANALYSIS

PROTEIN	52 GRAMS PER DAY
FAT	51 GRAMS PER DAY
CARBOHYDRATE	213 GRAMS PER DAY
KILOCALORIES	1565 KCAL PER DAY

Figure 1. Experimental diet.

seven of each week. Blood samples were collected by venipuncture at the Oklahoma State University student health facility between 0800 and 0830 A.M in 5 ml non-heparinized vacutainer tubes for whole blood and EDTA-treated tubes for serum analysis.

Serum was obtained by centrifuging whole blood at 1242 g at 5 C for 20 minutes. Serum was frozen (-20 C) in containers with 0.5 mg ascorbic acid added per 5 ml serum for later analysis. Whole blood was hemolyzed and diluted 1 to 9 with 0.1 % sodium ascorbate and frozen at -20 C. Hematocrits were measured immediately using whole blood in an Adams Autocrit Centrifuge.[3] All glassware used for sample storage and analysis was acid-washed in Chromerge solution and rinsed thoroughly with tap water and deionized, distilled water. Blood and folic acid solutions were handled in dim light and all analyses were performed under aseptic conditions.

SF and RBCF were determined by the method of Scott, et al (12). The test organism used was Lactobacillus casei, American Type Culture Collection (ATCC) No. 7469 which was obtained in freeze dried form. An actively growing culture was established using Bacto-Lactobacilli Medium.[4] Inocula for assay were prepared from a six-hour culture which was washed, centrifuged and decanted four times. One drop of

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[3]Clay Adams, Parsippany, N.J.

[4]Difco Laboratories, Detroit, MI

inoculum was dispensed per sample tube with a Pasteur pipette.

Folic acid standard solutions were prepared from folic acid diluted to 100 ng/ml.[5] Standard curves were prepared at folic acid concentrations of 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 ng/ml by dilution with 0.5% sodium ascorbate. These standard solutions were run with each assay. Triplicate samples of each serum and whole blood hemolysate sample were inoculated and incubated for 18 hours. Turbidity was read[6] after vortexing. Concentration of folate in all samples was calculated by regression based on turbidity vs log of PGA in the standards. RBCF was calculated by the formula:

$$\text{RBCF} = \frac{[\text{Whole Blood Folate}] - [\text{serum ng/ml X } (1 - \text{Hct}/100]}{\text{Hematocrit}/100}$$

Averages of triplicate assays were used for statistical analysis. Results were analyzed by analysis of variance and means were compared using Student's t test (13).

Results and Discussion

Mean SF values (Table 1) during week 1 exceeded the 8.1 ng/ml reported for women 20-44 years old in HANES II (7) by more than 30 percent while mean RBC folate was slightly

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[5]Sigma Chemical Company, St. Louis, MO

[6]Gilford Model 240 Spectrophotometer, Gilford Instrument Company, Oberlin, OH

Table 1. Scoring method for food folate checklist.

	Mean Folate Intake[2] ± SEM	Mean SF[3] ± SEM	RBCF ± SEM	HCT ± SEM
non-OCA	mcg/day	ng/ml	ng/ml	%
1-A	222 ± 78	22.3 ± 4.3	363	41
2-A	113 ± 43	13.4 ± 0.7	231	43
3-A	212 ± 105	8.2 ± 1.0	381	50
4-A	168 ± 74	21.4 ± 2.8	339	39
5-A	110 ± 78	5.5 ± 0.8	258	43
6-A	75 ± 19	4.3 ±(missing data)	188	42
	Mean SEM	Mean SEM	Mean SEM	Mean SEM
	150 ± 11	12.4 ± 1.5	293[a] ± 15.71	43 ±.75
OCA				
1-B	153 ± 27	19.3 ± 5.9	395	46
2-B	154 ± 65	9.2 ± 0.9	194	39
3-B	112 ± 67	7.1 ± 2.8	248	41.5
4-B	285 ± 138	7.5 ± 0.2	188	40
5-B	81 ± 50	2.5 ± 0.1	29	41.5
6-B	173 ± 53	8.5 ± 1.1	190	41
	Mean SEM	Mean SEM	Mean SEM	Mean SEM
	159 ± 13	9.0 ± 1.1	207[b] ± 23.58	41.4 ± .48

[a,b]Means in a column followed by different superscripts differ (P<.05).
[1]Mean SF on Day 3 = 12.4 ng/ml, Day 5 = 11.0 ng/ml, Day 7 = 10.1 ng/ml).
[2]Mean folate intake was averaged over 7 days.
[3]Mean SF was averaged over days 3,5 and 7.

below the 250 ng/ml reported in that survey (APPENDIX D). Despite a mean total folate intake during this period of only 155 mcg/day total folate, SF and RBCF levels remained normal. As analysis of the experimental diet exceeded its calculated folate content by 72 percent (74 vs 43 mcg/d) total folate content of the self-selected diets was probably closer to 265 than 155, mcg/day. Even that intake is only about two-thirds of the folate RDA. The discrepancy between calculated and analyzed total folate is similar to that noted by Chung et al (14), Pietarinen et al (10) and Moscovitch and Cooper (15). The calculated folate intakes agree with those calculated by others for a similar population group (16,17).

Daily food folate intake calculated from dietary records and published food folate values have consistently fallen below the RDA of 400 mcg/day total folate. Yet the incidence of low folate stores in the population is not correspondingly high. Bates and others (18) proposed that this discrepancy is a result of a combined effect of underestimation of food folate intake and overestimation of the dietary allowance for folate.

Mean SF values for days 3, 5 and 7 of week 1 were similar (Table 1) and masked individual variation. Although the mean for each day was well into the normal range, two of the 12 subjects, 3B and 5A, would be considered to have normal SF on one day but be at risk of folate deficiency on

another day. Herbert (19) and Edozien (20) documented similar problems with variation in SF values.

Mean SF and mean folate intake were not correlated among either OCA (R^2=.0299, P=.19) or non-OCA (R^2=.3788, P=.19) during the self-selected diet. RBCF increased (P<.01) as folate intake increased for non-OCA but not for OCA (Figure 2). Breskin et al (21) found that RBCF was related to intake among children and Dutram (22) noted that SF was correlated positively with dietary folate among adolescent girls. If the use of oral contraceptive agents interferes with folate metabolism, as proposed by Grace et al (23), RBCF would be expected to be more closely correlated with folate intake among non-OCA than among OCA.

Because SF responds to folate intake more rapidly than RBCF (24) the relationship of serum and RBC folate is of interest. Bailey et al (25) and Herbert et al (18) found that these two factors were closely related. In our trial, SF and RBCF were significantly correlated during the self-selected diet among all individuals (P<.007) and among OCA (P<.02) but not non-OCA (P<.24) (Figure 2). If oral contraceptives interfere with the absorption of folate, serum should vary less with daily intake among OCA. This could account for the closer relation between SF and RBCF among OCA than non-OCA.

SF changed rapidly and drastically in response to folate intake during the test periods. Mean SF was higher after seven days of the high folate diet than after seven

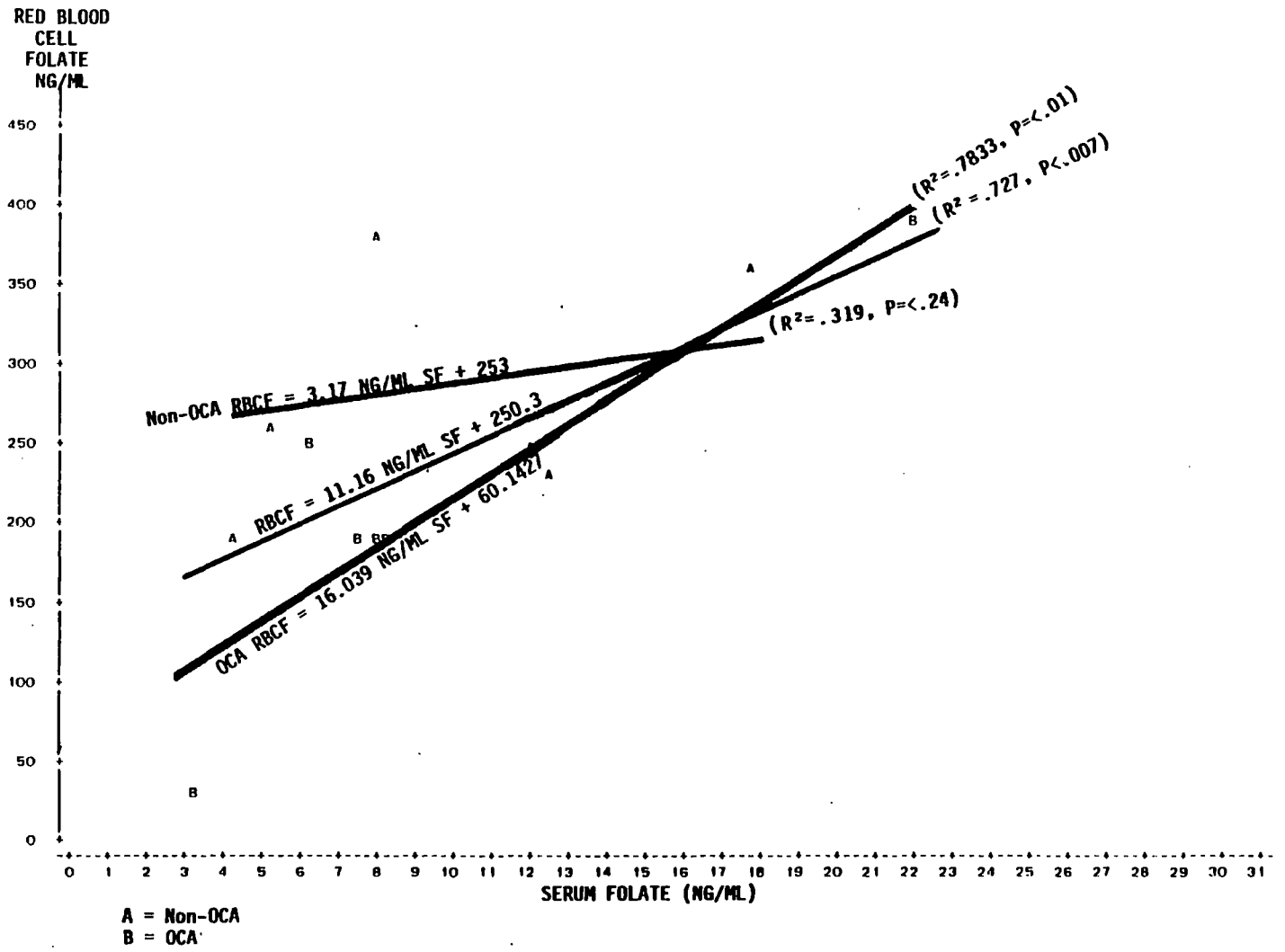


Figure 2. Correlation between RBCF and folate intake on self-selected diet based on contraceptive agent use.

days of the low folate diet (11.8 vs 8.0 ng/ml) (Table 2). This SF response agrees with results of other studies (4,5). Mean RBCF was not different after the high and low folate diets (239 vs 228 ng/ml) (Table 2). Figure 3 illustrates the changes in SF after 3,5 and 7 days on the low and high folate diets.

The concentration of folate in the basal diet used in this trial was similar to that of people who consume few fruits and vegetables whereas the supplemented diet provided 125 percent of the Folate RDA. Folate status, as reflected by SF concentration, responded within one week on each diet regimen. SF concentrations also differed among individuals. Had a crossover design not been employed, changes would not have been detected as being statistically significant.

The high-low diet order resulted in higher mean SF and RBCF levels (Table 3) than the low-high diet order (11.2 vs 8.6 and 264.7 vs 203 ng/ml, respectively). SF and RBCF differences between diets were more extreme during week 2 than week 3 (Table 4 and Figure 4) but not significantly so. This suggests that previous folate status may influence response to dietary folate intake. How many days of the second treatment would be needed to reach the more extreme SF and RBCF differences of the first treatment week (week 2) is not known.

Changes in SF and RBCF also were calculated as a percentage of the SF or RBCF for each individual between the self-selected diet and the end of the first treatment

Table 2. Mean SF and RBCF on high and low folate diets across treatment orders

Diet	Subjects	SF	RBCF
		(ng/ml)	(ng/ml)
High	12	11.8[a]	239
Low	12	8.0[b]	228

[a,b]Means followed by a different superscript (a,b) are significantly different ($P<.006$).

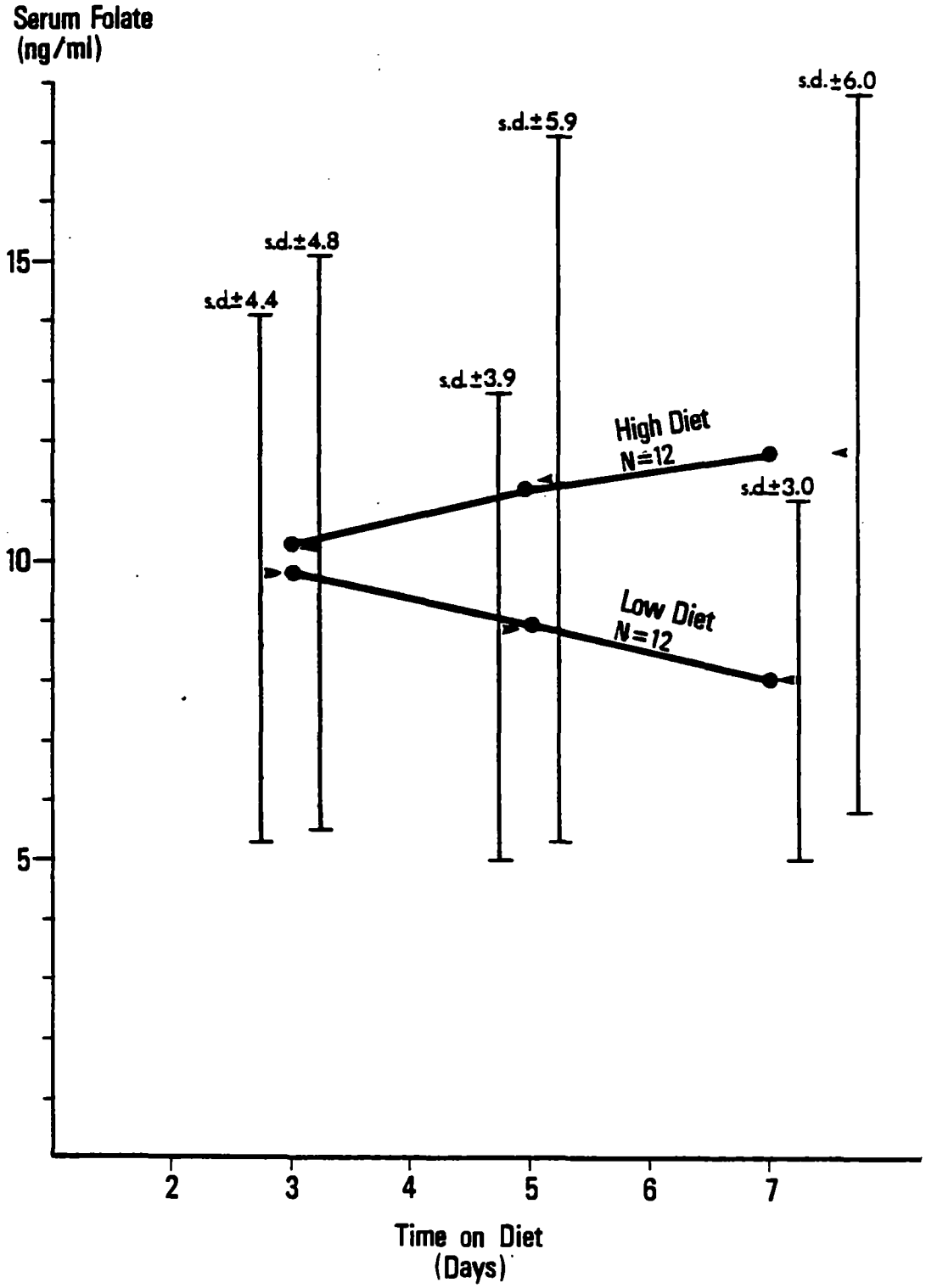


Figure 3. Mean SF on high and low folate diets.

Table 3. Effect of diet order on SF and RBCF across treatments

Diet Order	Subjects	Serum Folate (ng/ml)	RBC Folate (ng/ml)
High-Low	6	11.2[a]	264.7[c]
Low-High	6	8.6[b]	203.0[d]

[a,b]Means in a column followed by different superscripts differ ($P<.01$).

[c,d]Means in a column followed by different superscripts differ ($P<.002$).

Table 4. Effect of diet order on mean SF and RBCF

	Diet Order	Subjects	Week 1	Week 2	Week 3
			(ng/ml)	(ng/ml)	(ng/ml)
	Hi-Lo	6			
Serum Folate (ng/ml)			10.8	13.6	8.9
RBC Folate (ng/ml)			279.0	264.4	265.0
	Lo-Hi	6			
Serum Folate (ng/ml)			11.6	7.2	10.1
RBC Folate (ng/ml)			221.6	191.8	214.4

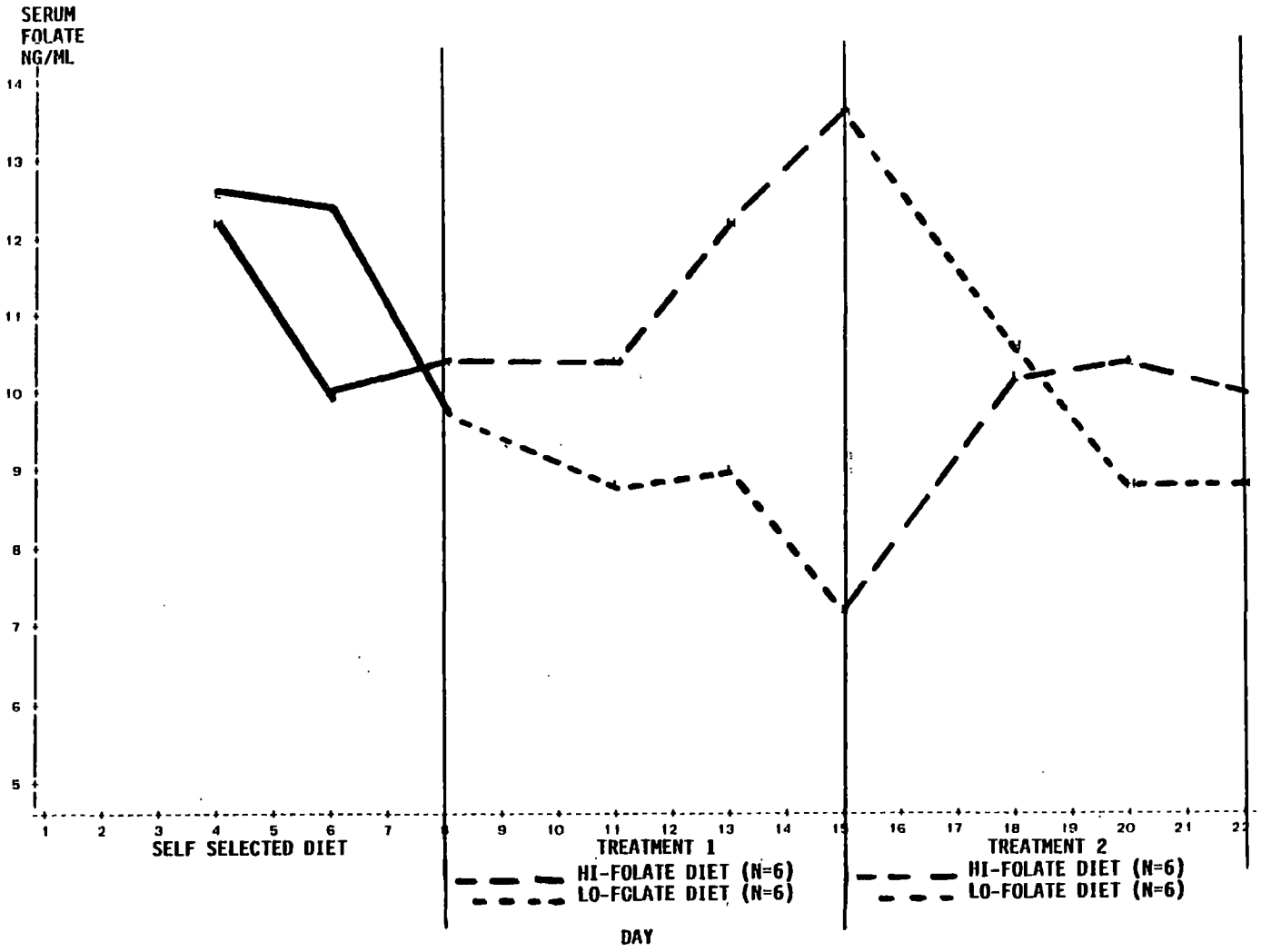


Figure 4. Mean SF on high-low and low-high diet order.

(week 2) (DSF1PC or DRBCF1PC) and from the end of the first treatment (week 2) to the end of the second treatment (week 3) (DSF2PC or DRBCF2PC). The percent change in SF and RBCF both differed ($P<.007$ and $P<.05$, respectively) on the high and low folate diets on both treatment orders (+51.4 vs -28.1 percent and +11.6 vs -0.7 percent for SF and RBCF, respectively on the high-low diet order and -30.8 vs +52.1 percent and -12.7 vs +16.8 percent for SF and RBCF, respectively on the low-high diet order) (Table 5). The percent SF change on each diet was similar regardless of diet order (Table 5). In contrast to the suggestion above, of a carryover effect, this indicates that the initial serum concentration does not influence the extent of change in SF in response to folate intake. Yet, the percent increase and decrease in RBCF were both somewhat greater for individuals on the low-high than the high-low diet order (Table 5)

Mean RBCF was lower ($P<.05$) among OCA than non-OCA at the start of the study (Table 1). Rhode et al (5) found SF to be lower among OCA in a large sample of a similar age group. HANES II (7) also reported that OCA use was associated with folate status but the effect was not significant among 20-44 year old females. SF and hematocrit did not differ with OCA use.

Mean SF concentration remained higher ($P<.02$) for non-OCA than OCA during the test weeks (Table 6). Figure 5 illustrates this difference over time. RBCF tended to be lower among OCA across treatments but the difference was not

Table 5 Mean percent increase and decrease in individual serum and RBC folate on high-low and low-high diet orders

Diet Order	N	Treatment 1		Treatment 2	
		Serum Folate (Percent Change)	RBC Folate (Percent Change)	Serum Folate (Percent Change)	RBC Folate (Percent Change)
High-Low	6	(+)51.4[a]	(+)11.6[c]	(-)28.1[b]	(-)0.7[d]
Low-High	6	(-)30.8[a]	(-)12.7[c]	(+)52.1[b]	(+)16.8[d]

[a,b]Means in a row followed by different superscripts differ ($P<.007$).

[c,d]Means in a row followed by different superscripts differ ($P<.05$).

Table 6. Effect of oral contraceptive use on SF and RBCF across treatments

OCA Use	N	Serum Folate	RBC Folate
		(ng/ml)	(ng/ml)
OCA	12	8.5[a]	218.4
non-OCA	12	11.4[b]	249.3

[a,b]Means in a column followed by different superscripts are significantly different ($P<.02$).

significant (Table 6). Rhode et al, (5) found no difference between SF of OCA and non-OCA with diets providing 159 mcg folate per day with or without 100 mcg supplemental folate. No interaction between OCA use and dietary treatment response in SF or RBCF was detected. The parallel lines for OCA and non-OCA across diets (Figure 5) indicates that their responses to treatments were similar.

The variation in SF, measured as the residual coefficient of variation, decreased steadily and consistently (R^2 = .8697, P<.002) from the self-selected diet to the end of the second treatment period (Figure 6). This indicates that the variation among individuals in SF which has been attributed by past researchers to physiological factors such as rate and extent of absorption, exchange with liver folate and other tissue stores, and rate of urinary excretion, may instead relate to variation in composition of the diet. As individuals consumed diets of similar composition over time, independent of the specific order of the diet, their folate levels became increasingly similar.

The relationships among dietary folate, RBCF and folate status are not well defined. Correlations among individuals during week 1 are presented in Figures 2 and 7. Folate intake of 50 percent of the RDA produced SF and RBCF concentrations in the normal range in our sample of adult women. Consumption of a standardized diet consistently decreased variability among individuals. Supplementation

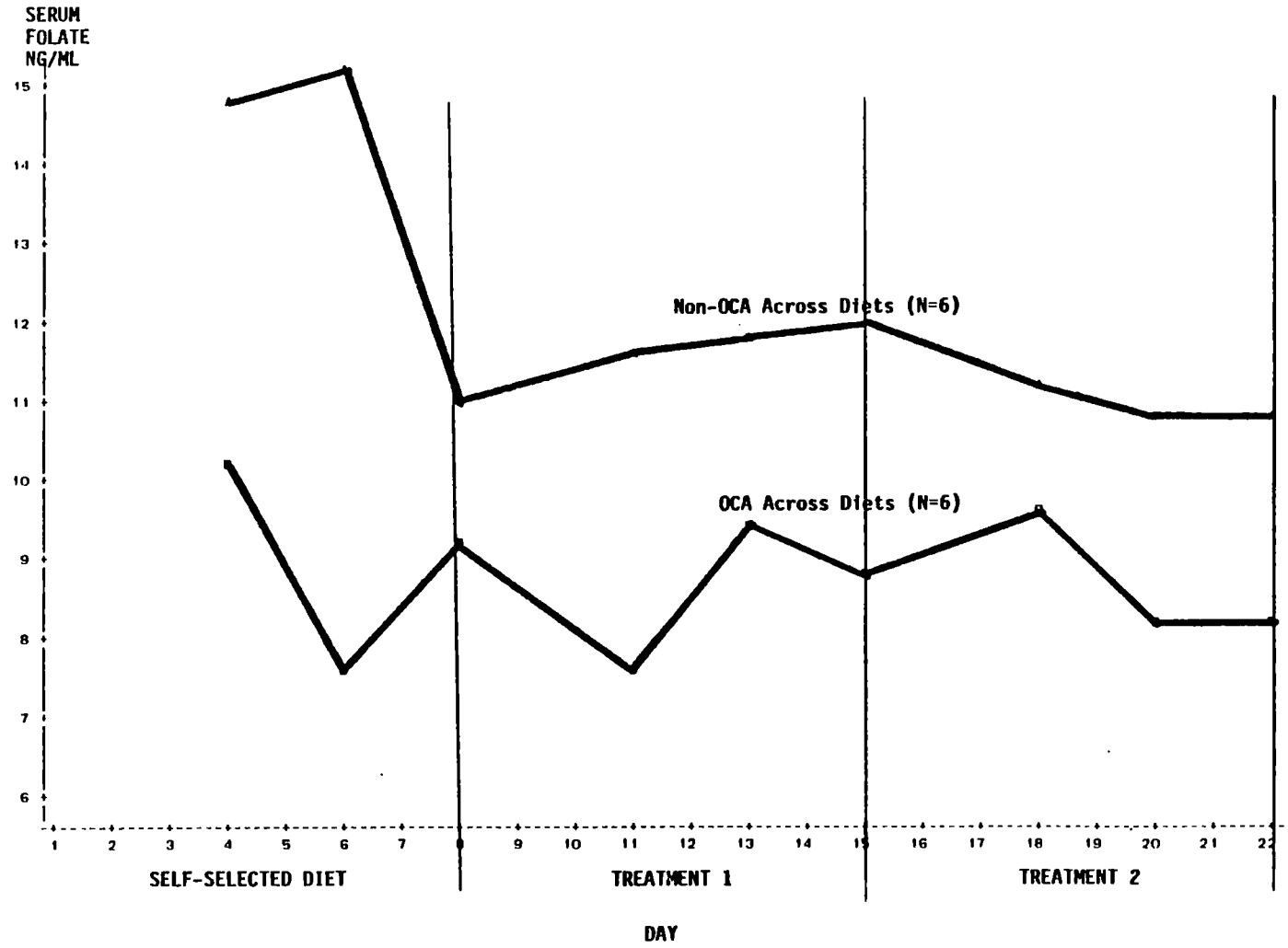


Figure 5. Mean SF of OCA and non-OCA.

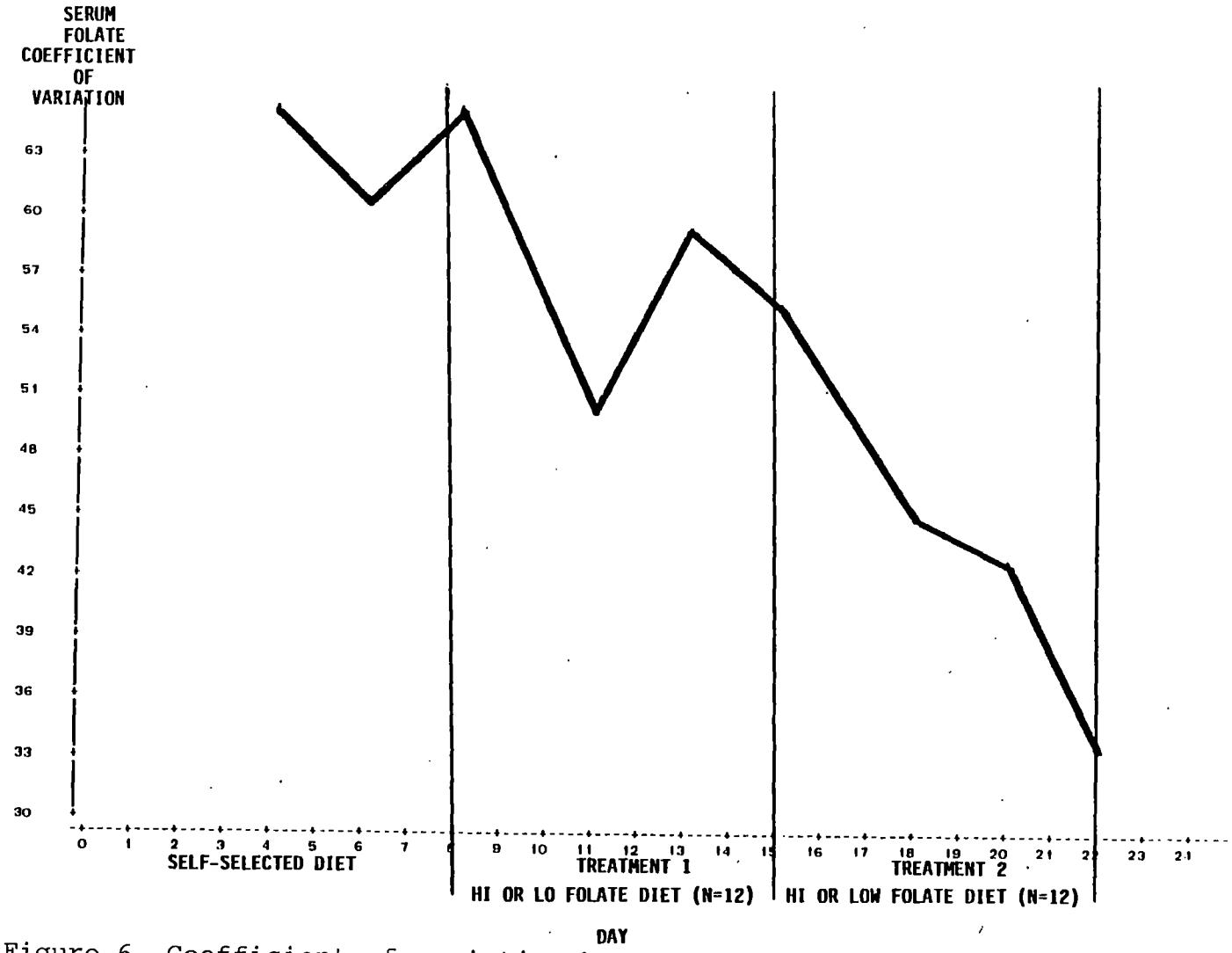


Figure 6. Coefficient of variation between subjects from beginning to end of study on self-selected and controlled folate diets.

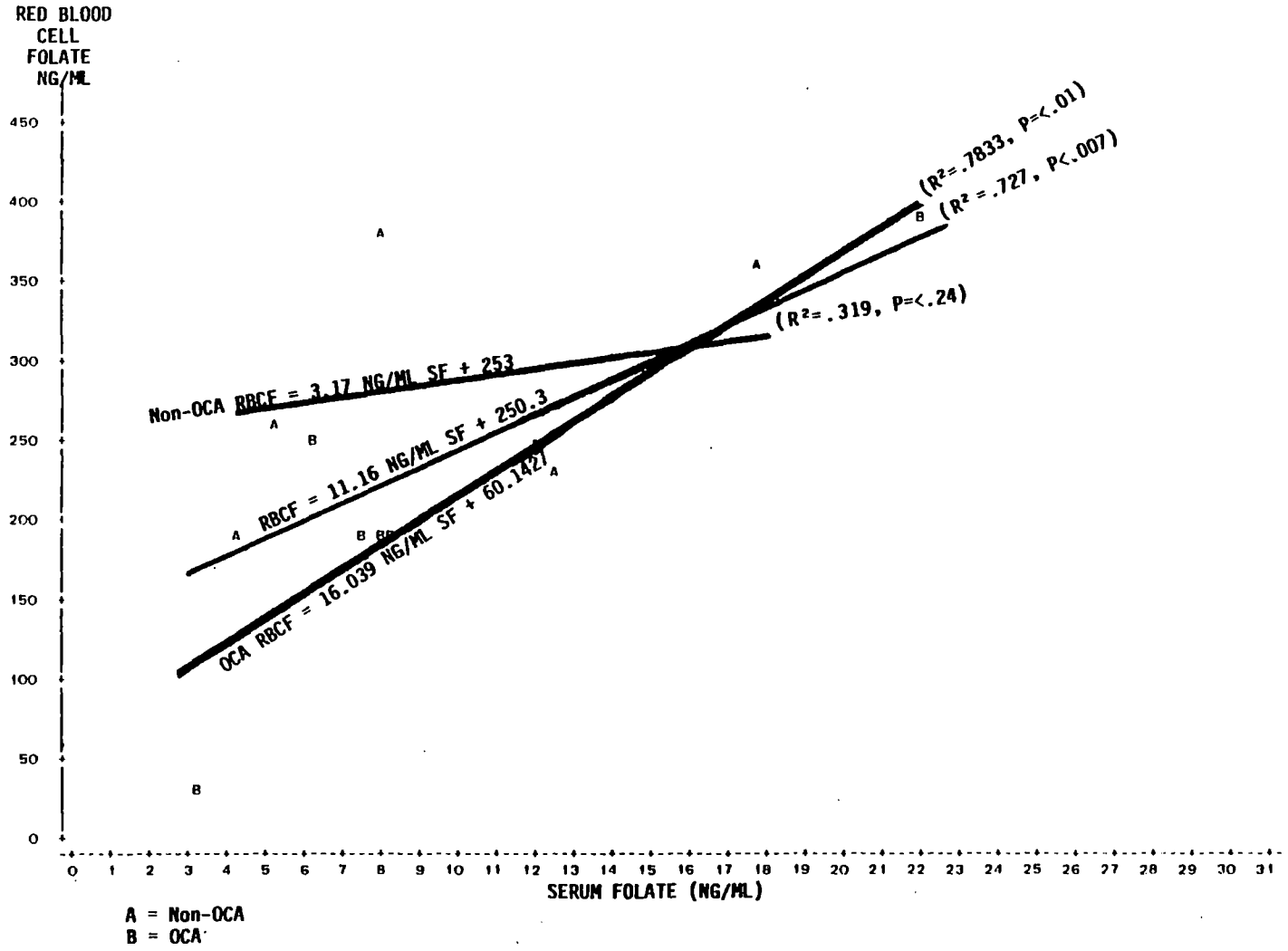


Figure 7. Correlation between initial RBCF and SF based on oral contraceptive use.

with 400 mcg folate daily increased SF concentrations. SF responses to folate intake were parallel among OCA and non-OCA, but SF of non-OCA was consistently higher throughout the study. Results of this experiment indicate that SF concentration varies with short term dietary changes. Hence serum measurement only assesses short-term folate status.

Literature Cited

1. Herbert, V. 1962b. Minimal daily adult folate requirement. Arch. Int. Med. 110:649-652.

2. Herbert, V. 1962a. Experimental nutritional folate deficiency in man. Trans. Assoc. Amer. Phys. 75:307 -320.

3. Herbert, V. 1962b. Minimal daily adult folate requirement. Arch. Int. Med. 110:649-652.

4. Eichner, E., Pierce, H., and Hillman, R. 1971. Folate balance in dietary-induced megaloblastic anemia. N. Engl. J. Med. 284:17. Apr. 29.

5. Rhode, B.M., Cooper, B.A., and Farmer, F.A. 1983. Effect of orange juice, folic acid, and oral contraceptives on serum folate in women taking a folate-restricted diet. J. Am. Coll. Nutr. 2:221-230.

6. Ten-State Nutrition Survey, 1968-1970. Part III Clinical, Anthropometry, Dental. U.S. Department of Health, Education and Welfare, Health Services and Mental Health Administration Center for Disease Control, Atlanta, Georgia. 1972.

7. Senti, F., and Pilch, S., editors. 1985. Analysis of folate data from the Second National Health and Nutrition Examination Survey (NHANES II). J. Nutr. 115:13989-1402.

8. Nutrition Canada. "Nutrition Canada National Survey." Ottawa: Information Canada. 1973.

9. Lan, S.J.: "Dietary folacin and serum folacin in adolescent girls." M.S. Thesis, Oklahoma State University, 1982.

10. Pietarinen, G.J., Leichter, J. and Pratt, R.F. Dietary folate intake and concentration of folate in serum and erythrocytes in women using oral contraceptives. 1977. Am. J. Clin. Nutr. 30:375-380.

11. Health-Aide User's Guide and Reference Manual. Corte Madera: Knossos, Inc., 1983.

12. Scott, J.M., Ghanta, V. and Herbert, V. Trouble-free microbiologic serum and red cell folate assays. 1974. Am. J. Med. Tech. 40:125-134.

13. Steel, R.G.D., and Torrie, J.H. Principles and Procedures of Statistics: A Biometrical Approach. Second Edition. New York: McGraw-Hill Book Company, 1980.

14. Chung, A.S., Pearson, W.M., Darby, W.J., Miller, O.N. and Goldsmith, G.A. 1961. Folic acid, vitamin B_6, pantothenic acid and vitamin B_{12} in human dietaries. Am. J. Clin. Nutr. 9:573.

15. Moscovitch, L.F. and Cooper, B.A. 1973. Folate content of diets in pregnancy: comparison of diets collected at home and diets prepared from dietary records. Am. J. Clin. Nutr. 26:707-714.

16. Yadrick, K.: "The effects of supplementation with zinc or zinc and iron on zinc, iron and copper status." Ph.D. Thesis, Oklahoma State University, 1986.

17. Sempos, C.T., Johnson, N.E., Smith, E.L. and Gilligan, C. 1984. A two-year dietary survey of middle-aged women: repeated dietary records as a measure of usual intake. J. Am. Dietet. Assoc. 84:1008-1013.

18. Bates, C.J., Black, A.E., Phillips, D.R., Wright, A.J. and Southgate, D.A. 1982. The discrepancy between folate intakes and the folate RDA. Hum. Nutr: Apl. Nutr. 36A:422-429.

19. Herbert, V., Colman, N., Spivack, M., Ocasio, E., Ghanta, V. Kimmel, K., Brenner, L., Freundlich, J. and Scott, J. 1975. Folic acid deficiency in the United States: Folate assays in a prenatal clinic. Am. J. Obstet. Gynecol. 123:175-179.

20. Edozien, J.C. 1972. National Nutrition Survey, Massachusetts. Report of the Survey Director to the Commissioner for Public Health, Commonwealth of Massachusetts, (Unpublished).

21. Breskin, M.W., Trahms, C.M., Worthington-Roberts, B., Labbe, R.F., and Koslowski, B. 1985. Supplement use: vitamin intakes and biochemical indexes in 40 to 108-month-old children. Am. J. Clin. Nutr. 85:49-56.

22. Dutram, K. "Serum folate status and dietary folacin intake of adolescent girls." M.S. Thesis, University of Arkansas, 1984.

23. Grace, E., Emans, S. and Drum ,D. 1982. Hematologic abnormalities in adolescents who take oral contraceptive pills. J. Pediatr. 101:771-774.

24. Sauberlich, H.E. "Detection of Folic Acid Deficiency in Populations." in Folic Acid Biochemistry and Physiology in Relation to the Human Nutrition Requirement. Food and Nutrition Board, National Research Council. Washington, D.C., 1977. National Academy of Sciences.

25. Bailey, L.B., Wagner, P.A., Christakis, G.J. and Davis, C.G. 1982. Folacin and iron status of adolescents from low-income rural households. Nutr. Res. 2(4): 397-407.

CHAPTER IV

A FOOD FOLATE CHECKLIST FOR MEASURING ADEQUACY OF FOLATE INTAKE

Abstract

A Daily Food Folate Checklist was compared with a 24-Hour Dietary Record for accuracy in measuring adequacy of total folate intake during one week. Twelve adult women recorded their food intake by both methods. There was a significant linear correlation ($P<.0001$) between daily folate intake calculated from the Dietary Records and Food Folate Checklist scores. Calculated folate intake varied significantly between subjects ($P<.0001$) over seven days while no significant differences in intake were evident between days. Mean calculated folate intake was 155 mcg total folate/day. The most frequently consumed folate-dense foods were breads, raw tomatoes, lettuce, nuts and eggs. Correlation between folate intake calculated from 24-Hour Dietary Records and Food Folate Checklist scores indicates that such a checklist is a viable instrument for estimating adequacy of folate intake.

Resume

A Food Folate Checklist was compared with a 24-Hour Dietary Record for measuring adequacy of folate intake among adult women. There was a significant linear correlation ($R = .9059$, $P<.000$) between daily folate intake calculated from Food Folate Checklist scores and Dietary Records. The Checklist is a viable alternative to the Food Record for measuring dietary folate adequacy.

Introduction

Serum folate responds rapidly to changes in dietary folate and reflects recent folate intake. In various trials, serum folate reached deficient concentrations in less than one week (1) and in four weeks (2) on folate deficient diets and decreased significantly within the normal range in one week (3) and two weeks (4) on diets providing 74 mcg and 159 mcg total folate/day, respectively. Serum folate has been used as a measure of folate status either by itself (5,6) or in conjunction with red blood cell folate (7,8). Because serum folate changes rapidly in response to diet, a single measurement has limited use for detecting a deficiency in the absence of a dietary intake record.

Until recently the folate content of individual foods was substantially underestimated due to assay problems. More accurate published tables of food folate content are now available (9,10,11,12,13). Calculations of folacin

intake from current food tables, however, still can be somewhat below analyzed values for individual foods (14, 15).

Folate is concentrated in a relatively small number of foods, mostly fruits and vegetables. For this reason food frequency questionnaires have been used to relate folate status (16) or folate intake (17) to the consumption of particular categories of foods or certain food items. This study was designed to compare the use of a daily Food Folate Checklist which includes only the rich sources of folate, with a daily 24-Hour Dietary Record to evaluate the adequacy of folate intake during a one week period.

Methods

The study sample included twelve adult females, 20-41 years old, who were participating in a diet study measuring short-term changes in serum folate in response to dietary changes. The subjects consumed a self-selected diet, excluding nutrient supplements, for one week. They were instructed to estimate their food intake using food models and measuring utensils. All subjects completed a 24-hour dietary record and a Food Folate Checklist daily.

The Food Folate Checklist is a listing of 26 foods each of which provides at least 25 mcg total folate/100 grams (11). Unlike a food frequency questionnaire, the Food Folate Checklist (Figure 1) was designed to be completed daily. Foods were divided into four groups based on the

SUBJECT NUMBER_____ DAY OF WEEK _____ DATE_____

FOOD FOLATE CHECKLIST

PLEASE INDICATE WITH A CHECK MARK, WHICH OF THE FOLLOWING FOODS YOU ATE TODAY, HOW MANY TIMES DURING THE DAY YOU ATE EACH ONE, WHETHER IT WAS RAW OR COOKED AND THE AMOUNT YOU ATE.

			NUMBER OF TIMES EATEN	SERVING SIZE		
	RAW	COOKED		A FEW BITES	1/4 CUP OR MORE	OTHER MEASURE
Bananas	1					
Orange Juice	3					
Tomato Juice	2					
Lettuce	1					
Cabbage	1					
Tomatoes	2	1				
Carrots	1					
Cauliflower	1					
Broccoli	3	3				
Spinach	3	3				
Beets		2				
Yellow or Wax Beans		1				
Green Beans		1				
Brussels Sprouts		1				
Kidney (red) Beans		2				
Pinto (brown speckled) Beans		2				
Avocado	1					
Eggs		3				
Peanut Butter	1					
Nuts - Kind _____	2					
Liver - Kind		3				
Bread - Kind	1					
Cold or Hot Cereal Instant Breakfast Drinks or Bars Brand Name_____	1 4	1 4				
Wheat Bran	3					
Wheat Germ	3	3				
Brewers Yeast	3					

Group 1 - 5-20 mcg total folate/serving (2.5 points/serving)
(includes non-fortified cereals)
Group 2 - 20-30 mcg total folate/serving (5 points/serving)
Group 3 - 31-70 mcg total folate/serving (10 points/serving)
Group 4 - 100 mcg total folate/oz. (fortified cereals & breakfast bars)
(20 points/serving)

Figure 1. Food folate checklist.

amount of total folate per 1/4 cup serving unless another portion size was considered customary (e.g. total folate per 1/2 cup was used for juices and total folate per one whole egg was used for eggs). All foods in each group are assigned the same numerical score based on their average folate content per one-fourth cup serving. The scoring system is shown in Table 1. The serving size category "a few bites" is counted as one-half serving and is designed to include the folate contribution of raw vegetables commonly served at salad bars and eaten in small quantities. The serving size category "other measure" is intended to aid in recording food items such as bananas and slices of bread which are not commonly measured in terms of cups. The Checklist distinguishes between raw and cooked foods since folate is water soluble and labile to heat. In some cases, only the raw food contains enough folate to be included in the scoring.

The scoring method is based on one point per 5 mcg total folate. A score of 80 is equal to the adult folate RDA of 400 mcg.

The nutrient content of the 24-hour dietary records, including total folate, was calculated using the Health Aide (18) microcomputer program. Calculated folate intake and Food Folate Checklist scores were compared by the Pearson r correlation. Variation in folate intake from day to day was measured by analysis of variance of folate intake among subjects for different numbers of days of the week.

TABLE 1

Scoring Method for Food Folate Checklist

Food Group	Foods in Group	Range of Total Folate Per Serving	Average Total Folate Per Serving	Points Assigned Per Serving	Percent of Folate RDA Per Serving
		Mcg	Mcg		Percent
1	Bananas, Lettuce, Cabbage Cooked Tomatoes, Raw Carrots, Raw Cauliflower, Yellow or Wax Beans, Green Beans, Brussels Sprouts,, Avocado, Peanut Butter, Bread, Non-fortified break-fast Cereals,	5 - 20	12.5	2.5	3
2	Tomato Juice, Raw Tomatoes, Beets, Kidney Beans, Pinto Beans, Nuts	20 - 30	25	5	6
3	Orange Juice, Raw and Cooked Broccoli, Raw and Cooked Spinach, Eggs, Liver, Wheat Bran, Wheat Germ, Brewers Yeast	30 - 70	50	10	12.5
4	Cold and Hot Forti-fied Cereals	100	100	20	25

Vitamin C and Vitamin A intake were compared to folate intake since they also would be expected to vary in relation to consumption of fruits and vegetables.

Results

Table 2 shows the rank order of consumption of foods on the checklist from most to least frequently consumed, the number of times each food was selected and the total number of servings of each food consumed. The five most frequently consumed folate sources were: breads (including tortillas, pizza crust and crackers) raw tomatoes, lettuce, nuts and eggs. The five least frequently consumed sources of folate were kidney beans, tomato juice, avocado, spinach and broccoli. Folate-rich foods which were not consumed by these 12 subjects were brewers' yeast, wheat bran, liver, brussels sprouts and wax beans.

The percent contribution of each food to the total folate intake is also shown in Table 2. The five highest contributors to folate intake were: breads, orange juice, raw tomatoes, lettuce and eggs. When foods in the Checklist were considered as groups according to the Basic Four Food Groups (Table 2) the fruit and vegetable group contributed 50 percent of the total folate intake of the 12 subjects. The bread and cereal group contributed 30 percent and the meat and meat alternate group contributed 20 percent. No foods in the dairy group were included in the Checklist. Similar orders of food group contributions to total folate

Table 2. Frequency of selection of foods in checklist.

Food item	Number of times selected	Number of servings	Per cent contribution to folate intake
			%
Breads	83	133	14.0
Raw tomatoes	43	60	12.5
Lettuce	39	105	11.0
Nuts	27	22	4.5
Eggs	21	27	11.0
Orange juice	18	34	14.0
Peanut butter	17	21	2.0
Carrots	9	10	1.0
Cabbage	8	10	1.0
Pinto beans	8	11	2.0
Cauliflower	7	7	<1.0
Non-fortified cereal	9	7	1.0
Fortified cereal	6	10	8.0
Fortified breakfast bars	6	7	6.5
Green beans	6	12	1.0
Broccoli	4	8	3.0
Bananas	4	12	1.0
Spinach	3	4	2.0
Tomato juice	3	3	<1.0
Avocado	2	5	<1.0
Beets	1	1	<1.0
Kidney beans	1	3	<1.0

Foods never selected
Liver
Brussels sprouts
Wheat bran
Wheat germ
Brewers yeast
Yellow or wax beans

Contribution of food groups to folate intake	
Breads/cereals	29%
Fruits/Vegetables	51%
Meats/Alternates	20%

intake were reported by Spring et al (1979) and Poh Tan (1984) for the UK. Hoppner et al (1972), on the other hand, reported that in Canada meat, fish and poultry provided more folate than vegetables or cereal products. This folate distribution appears to be attributable to the distribution of total food quantities consumed in each group by the population.

The mean total folate intake as calculated from the 24-hour Dietary Records for one week was 155 mcg total folate/day (± 17.97 SEM) which is less than 50 percent of the RDA. The mean Food Folate Checklist score was 30.2 (± 4.25 SEM) (Table 3) which is equivalent to 151 mcg of folate. A significant linear correlation ($R = .9059$, $P<.0001$) was detected between mcg of total folate intake calculated from the 24-hour dietary records and the Food Folate Checklist scores (Figure 2). Vitamin C and Folate intake (Table 3) also were significantly correlated ($R = .7742$, $P<.003$, Figure 3). Calculated Vitamin A and Folate intake were not significantly correlated though Vitamin C and A intakes were correlated with each other ($P<.002$, $R = .7960$) (Appendix E).

Folate intake varied significantly and consistently between individuals whether based on intake from only two days ($P<.0007$) or as many as seven days ($P<.0001$) (Appendix F). The wide variation in mean folate intake between individuals is shown in Figure 4. Mean folate intake did

Table 3. Mean folate intake, Vitamin C intake and Food Folate Checklist score on self-selected diet

Subject	Mean folate intake ± SEM	Mean Vitamin C intake ± SEM	Mean Food Folate Checklist Score ± SEM
	mcg/day	mg/day	
1-A	222 ± 78	132 ± 51	39 ± 11
2-A	113 ± 43	37 ± 45	27 ± 13
3-A	212 ± 105	48 ± 27	48 ± 27
4-A	168 ± 74	153 ± 101	23 ± 13
5-A	110 ± 79	69 ± 60	18 ± 14
6-A	75 ± 19	47 ± 39	16 ± 7
1-B	153 ± 27	153 ± 43	21 ± 9
2-B	154 ± 65	71 ± 55	35 ± 20
3-B	112 ± 65	65 ± 48	29 ± 19
4-B	285 ± 138	146 ± 103	61 ± 22
5-B	81 ± 51	63 ± 24	8 ± 6
6-B	173 ± 53	92 ± 18	38 ± 8
	Mean SEM	Mean SEM	Mean SEM
	154 ± 18	90 ± 13	30 ± 4

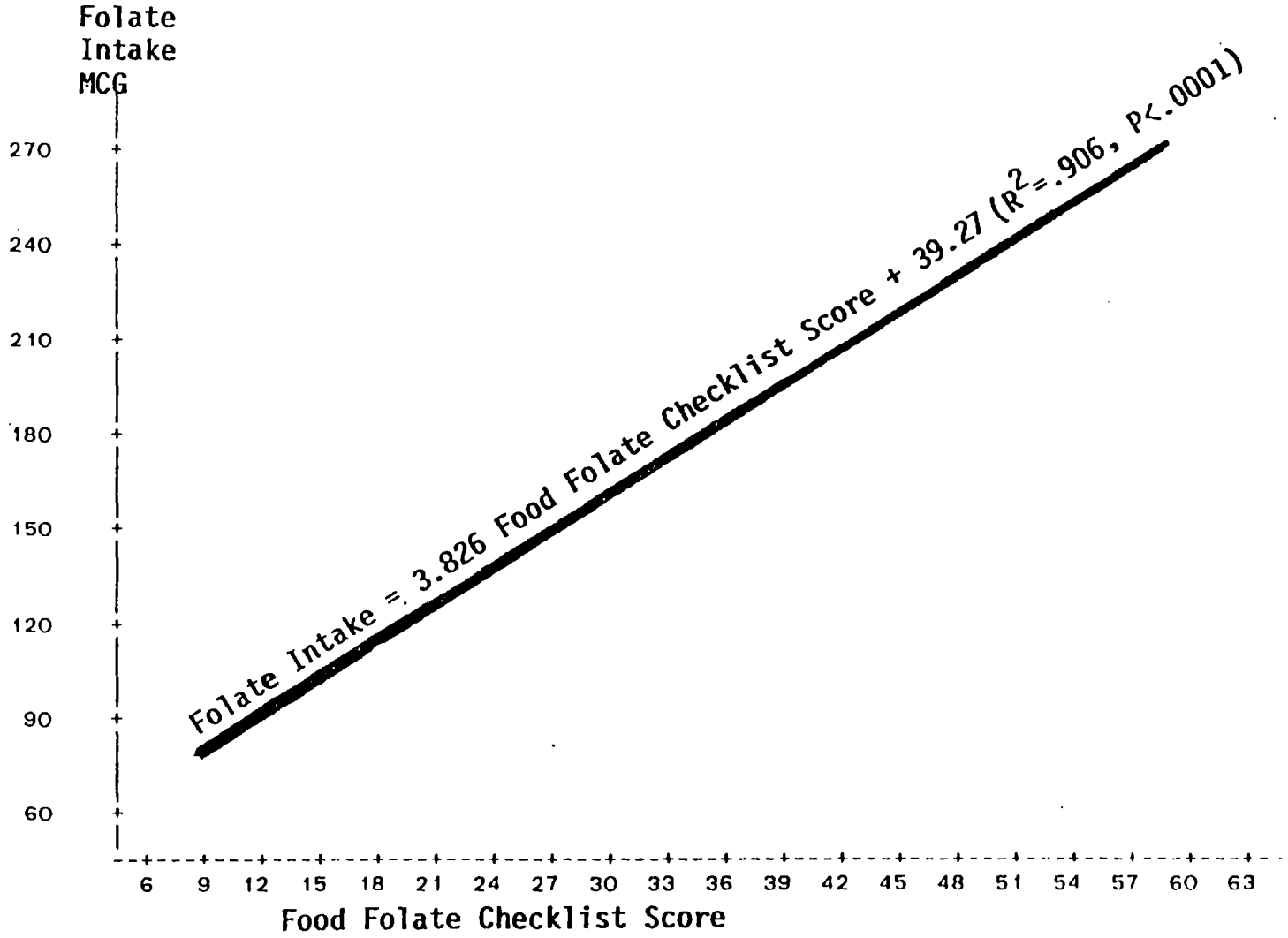


Figure 2. Correlation between folate intake and Food Folate Checklist score.

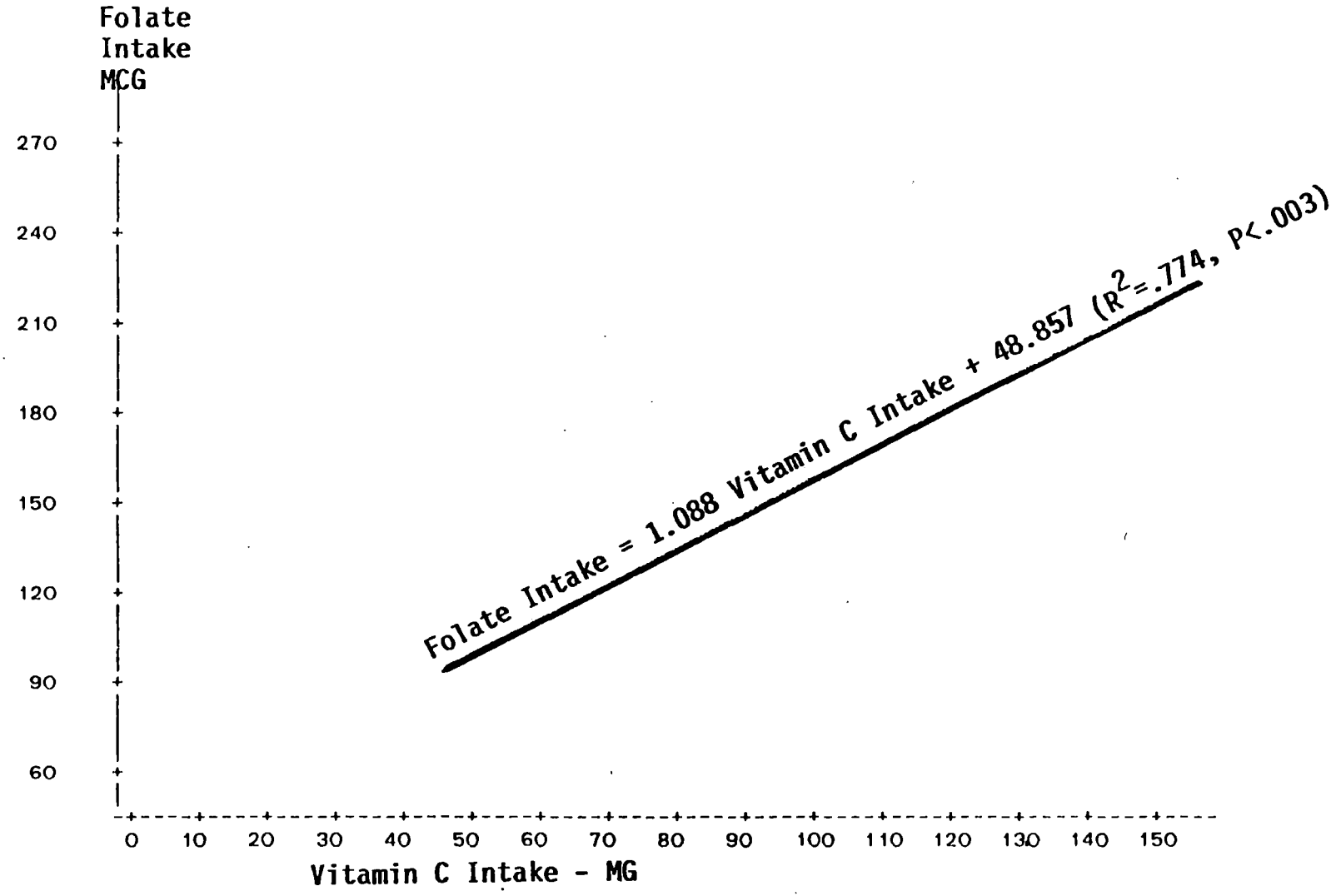


Figure 3. Correlation between folate intake and vitamin C intake.

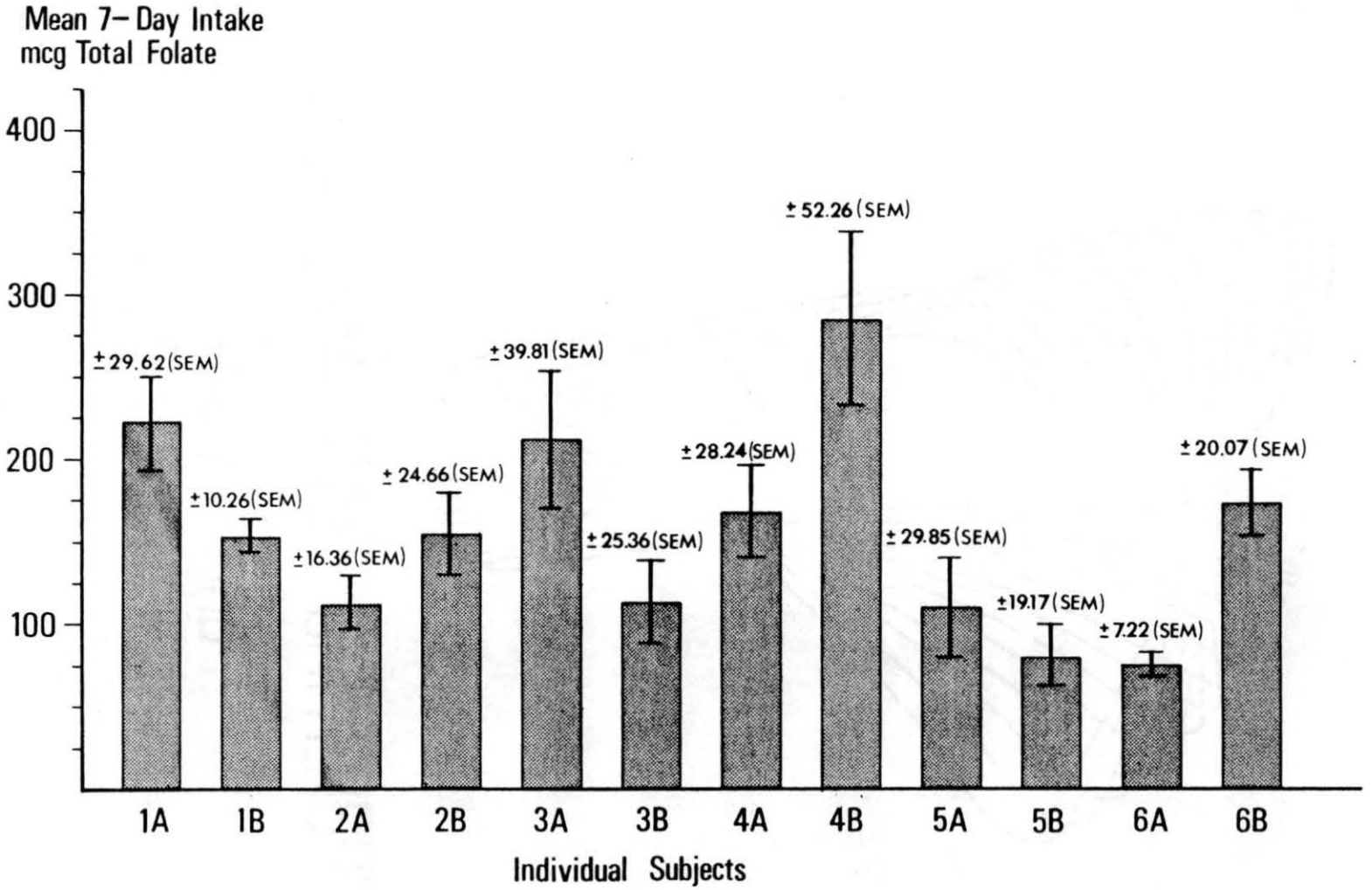


Figure 4. Mean folate intake of individuals for 7 days.

not vary between days. The consistency of folate intake between days is shown in Figure 5.

Vitamin C intake also differed ($P<.04$) between subjects though this was first detected only after four days, and increased over time to day seven ($P<.0003$). Significant differences between individuals in Vitamin A intake were not evident until after five days ($P<.03$) and the significance level did not increase by including data up to seven days ($P<.04$). These data indicate that folate intake is more habitual for individuals than is intake of either Vitamin C or Vitamin A. This may be attributable to the limited number of foods which are good folate sources and personal preferences with regard to those foods.

In this trial serum folate was not significantly correlated with either calculated folate intake or Food Folate Checklist score during one week. The highest mean weekly folate intake was less than 75 percent of the folate RDA, yet all but two subjects had normal serum folate values. Only two subjects had mean folate intakes below 25 percent of the folate RDA. One of these had a deficient red blood cell folate concentration (<140 ng/ml) and both had low (3.0 - 5.9 ng/ml) serum folate concentrations.

Discussion

Breads ranked first among folate sources in frequency of consumption although the category "breads" included a much wider variety of foods than other categories.

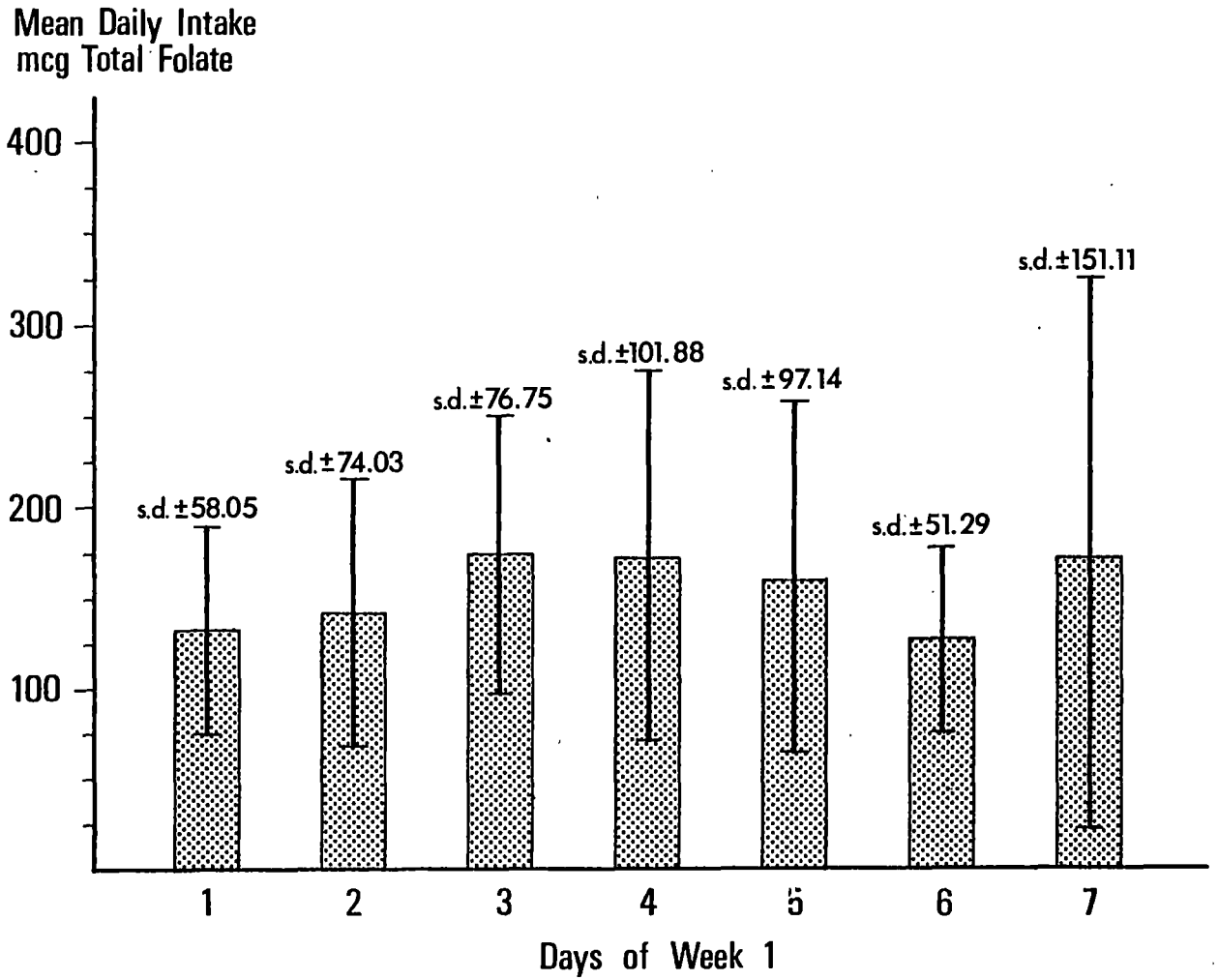


Figure 5. Mean daily folate intake for 12 individuals.

Frequency of consumption of a food item does not necessarily indicate the magnitude of its contribution to the day's folate intake. A single one ounce serving of a fortified cereal or one cup of raw spinach each provide one-fourth (100 mcg) of the folate RDA. In comparison, eight slices of whole wheat bread are needed to provide an equal amount of folate. The food's folate concentration needs to be considered. Table 2 illustrates that 133 servings of bread and only 34 servings of orange juice each contributed 14 percent to total folate intake for one week. The correlation between Vitamin C intake and folate intake indicates a sizable folate contribution from fruits and vegetables which are also rich sources of vitamin C.

Folate intake was consistent with other calculations of folate intake among similar populations (19,20). Serum folate was not significantly related to calculated folate intake in this study. The fact that the two subjects with the lowest folate intake also had the lowest blood folate levels suggests that the relationship between serum folate and folate intake may be close. The strong correlation between dietary folate calculated from the 24-hour dietary record and the Food Folate Checklist scores indicates that the Checklist may be a useful alternative for the 24-hour record when folate is the nutrient of interest. One might obtain a folate intake estimate over a one week period using the Checklist which would correct for day-to-day folate intake fluctuation.

The consistent folate intake by each individual over time reflects individual dietary patterns in folate intake. The absence of differences in folate intake on different days also reflects consistency in individual intake over the one week period. The variation in mean folate intake between individuals over seven days was greater than the variation between the days of the week for all individuals. As folate intake appears to be consistent for individuals over time, one or two days' Food Folate Checklists from each of two or three weeks (to account for variation in groceries purchased from week to week) should give a reliable picture of individual folate intake. Serum folate measurement would be a more reliable indicator of folate status if it were associated with information about the consistency of folate intake during recent days. The Food Folate Checklist is a simple instrument for collection of such dietary data.

References

(1) Eichner, E.R., and Hillman, R.S.: Effect of alcohol on serum folate level. J. Clin. Invest. 52:584, 1973.

(2) Herbert, V.: Minimal daily adult folate requirement. Arch. Int. Med. 110:649, 1962.

(3) Georgiou, C.: "Dietary folate and serum folate levels." Ph.D. Thesis, Oklahoma State University, 1986.

(4) Rhode, B.M., Cooper, B.A., and Farmer, F.A.: Effect of orange juice, folic acid, and oral contraceptives on serum folate in women taking a folate-restricted diet. J. Am. Col. Nutr. 2:221, 1983.

(5) Lan, S.J.: "Dietary folacin and serum folacin in adolescent girls." Unpublished M.S. Thesis, Oklahoma State University, 1982.

(6) Nutrition Canada. "Nutrition Canada National Survey." Ottawa: Information Canada. 1973.

(7) Senti, F.R., and Pilch, S.M.: "Assessment of the folate nutritional status of the U.S. population based on data collected in the second National Health and Nutrition Examination Survey, 1976-1980." Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Maryland. 1984.

(8) Ten-State Nutrition Survey, 1968-1970. Part III - Clinical, Anthropometry, Dental. U.S. Department of Health, Education and Welfare, Health Services and Mental Health Administration Center for Disease Control, Atlanta, Georgia. 1972.

(9) Hoppner, K., Lampi, B., and Perrin, D.E.: Free and total folate activity in foods available on the Canadian market. J. Can. Inst. Food Sci. Technol. 5a;60, 1972.

(10) Hoppner, K., Lampi, B. and Smith, D.: "Data on Folacin activity in Foods: Availability, Applications and Limitations." In Folic Acid: Biochemistry and Physiology in Relation to the Human Nutrition Requirement. National Research Council, National Academy of Sciences. Washington, D.C., 1977.

(11) Perloff, B.P., and Butrum, R.R.: Folacin in selected foods. J. Am. Dietet. Assoc. 70:161, 1977.

(12) Santini, R., and Corcino, J.J.: Analysis of some nutrients of the Puerto Rican diet. Am. J. Clin. Nutr. 27:840, 1974.

(13) Spring, J.A., Robertson, J., and Buss, D.H.: Trace nutrients. magnesium, copper, zinc, vitamin B_6, vitamin B_{12} and folic acid in the British household food supply. Br. J. Nutr. 41:487, 1973.

(14) Chung, A.S., Pearson, W.M., Darby, W.J., Miller, O.N., and Goldsmith, G.A. Folic acid, vitamin B_6, pantothenic acid and vitamin B_{12} in human dietaries. Am. J. Clin. Nutr. 9:573, 1961.

(15) Pietarinen, F., Leichter, J., and Pratt, R.: Dietary folate intake and concentration of folate in serum and erythrocytes in women using oral contraceptives. Am. J. Clin. Nutr. 30:375, 1977.

(16) Bailey, L.B., Wagner, P.A., Davis, C.G., and Dinning, J.S.: Food frequency related to folacin status in adolescents. J. Am. Dietet. Assoc. 84:801, 1984.

(17) Dutram, K.: "Serum folate status and dietary folacin intake of adolescent girls." M.S. Thesis, University of Arkansas, 1984.

(18) Health-Aide User's Guide and Reference Manual. Corte Madera: Knossos, Inc., 1983.

(19) Spring, J.A., Robertson, J. and Buss, D.H. 1979. Trace nutrients. Magnesium, copper, zinc, vitamin B_6, vitamin B_{12} and folic acid in the British household food supply. Br. J. Nutr. 41: 487-493.

(20) Poh Tan, S., R.W. Wenlock and D.H. Buss. 1984. Folic acid content of the diet in various types of British household. Hum. Nutr.: Appl. Nutr. and Clin. Prac. 38A:17-22.

(21) Hoppner, K., Lampi, B. and Perrin, D.E. 1972. Free and total folate activity in foods available on the Canadian market. J. Can. Inst. Food Sci. Technol. 5:60-66.

(22) Sempos, C.T., Johnson, N.E., Smith, E.L., and Gilligan, C.: A two-year dietary survey of middle-aged women: repeated dietary records as a measure of usual intake. J. Am. Dietet. Assoc. 84:1008, 1984.

(23) Yadrick, K.: "The effects of supplementation with zinc or zinc and iron on zinc, iron and copper status." Ph.D. Thesis, Oklahoma State University, 1986.

CHAPTER V

SUMMARY AND RECOMMENDATIONS

Summary

This dissertation evaluated the effect of consumption of a low and high folate diet on serum and red blood cell folate (SF and RBCF) among young adult women. Oral contraceptive agent users (OCA) and non-users (non-OCA) and diet order were compared.

A Food Folate Checklist also was evaluated as an instrument for measuring the adequacy of folate intake on a self-selected diet. Folate intake calculated from the Checklist and from 24-hour dietary records were compared for seven days.

The study was based on eight hypotheses. Each will be discussed separately. Then general conclusions and recommendations for further research will be addressed.

Hypothesis one was that individual mean fasting SF would be positively associated with individual mean daily folate intake among adult women consuming a self-selected diet during one week. No correlation was found between SF and folate intake but RBCF, which represents folate tissue stores, was positively correlated with dietary folate among non-OCA subjects. This indicates that folate status is

related to folate intake. If oral contraceptive agents interfere with folate metabolism, then the folate status should be more closely related to folate intake of non-OCA than of OCA subjects.

Mean folate intake on the self-selected diet was estimated to be only about two-thirds of the folate RDA, yet mean SF was well into the normal range. This finding is consistent with those of large population studies which have led to the recommendation that the folate RDA be lowered. Mean SF masked low individual folate levels. This observation supports previous suggestions that SF values should be considered individually for purposes of nutritional status assessment.

Total folate intake on a self selected diet was consistent within individuals from day to day during one week. Folate intake was significantly different between individuals even when as few as two days were compared. These data imply that folate intake is highly individual and is habitual from day to day.

Hypothesis two was that restricting folate intake to 50 mcg total folate per day would significantly lower fasting SF in one week or less. The low folate diet, as analyzed, provided 74 mcg total folate per day. Fasting SF was lowered by an average of 29.5 percent in one week on the folate restricted diet. Although variation between individuals was observed mean SF declined steadily from day 3 to day 7 for subjects on the restricted diet.

Hypothesis three was that the decrease in SF on the low folate treatment would be inversely proportional to initial fasting SF following consumption of a self-selected or high folate diet for one week. SF decreased on the low folate diet by nearly the same percentage when it followed the high folate as when it followed the self-selected diet (30.8 vs 28.1 percent, respectively). Mean SF was lower after one week on the low folate diet when it was preceded by the self-selected diet than by the high folate diet. This was a function of the lower starting SF after the self-selected than after the high folate diet. This result indicates that previous SF level does not affect the extent to which SF decreases on a folate restricted diet. Rather, folate intake is the primary determinant of SF decrease.

Hypothesis four was that a folate supplement of 400 mcg PGA per day would increase fasting SF in one week or less. Mean SF increased 51 percent in seven days of the folate supplemented diet (474 mcg total folate/d). This SF increase with a folate supplement was greater than the decrease in SF with folate restriction. Individual variation was greater on the supplemented than on the restricted diet, but mean SF increased steadily from day 3 to day 7 of supplementation.

Hypothesis five was that the increase in fasting SF on the high folate treatment would be inversely proportional to initial fasting SF following consumption of a self-selected diet or a low folate diet for one week. SF increased on the

high folate diet by almost exactly the same amount after one week on the self-selected diet (51 percent) and the low folate diet (52 percent). SF reached a higher concentration in 7 days of folate supplementation when it followed the self-selected than when it followed the low folate diet as a function of the higher starting point. In this case also, folate intake, not prior SF concentration, was the primary factor influencing the extent of increase in SF with supplementation.

Hypothesis six was that there would be no difference between initial fasting RBCF and RBCF after consuming a low folate or high folate diet for one week, regardless of treatment order. No difference was observed between RBCF after one week of the high and low diet. Diet order did, however, have a significant affect on RBCF. The high-low diet order produced a higher RBCF across treatments than did the low-high diet order.

The percent increase in RBCF on the high folate diet was greater than the percent decrease on the low folate diet regardless of diet order. This indicates that RBCF responds more rapidly to dietary folate when dietary folate increases than when it decreases. Unlike SF, RBCF decreased on the low folate diet more than 18 times as much when it followed the self-selected diet than when it followed the high folate diet. RBCF increased about 30 percent more on the high folate diet when it followed the low folate than when it followed the self-selected diet. RBCF appears to respond

more to a change in folate intake (either a low or high folate diet) when that change follows a period of low folate intake.

Hypothesis seven was that folate status is influenced by use of oral contraceptive agents. SF was not significantly different between OCA and non-OCA during the week of a self-selected diet though RBCF was significantly lower among OCA. Folate intake was similar among the two groups. Mean SF during the high and low folate diets was lower among OCA than non-OCA but RBCF was not. These data support previous evidence that oral contraceptive agents lower indices of folate status.

Hypothesis eight was that daily total folate intake calculated from a Food Folate Checklist which included only significant food sources of folic acid would correlate positively with folate intake calculated from 24-hour dietary records. Food Folate Checklist scores correlated positively with folate intake calculated from 24-hour dietary records. This finding indicates the Checklist can be substituted for a 24-hour dietary record to calculate folate intake. Folate intake also was positively correlated with Vitamin C intake as might be expected becase they are both present in many of the same foods.

A hypothesis was not proposed regarding the relationship between SF and RBCF. SF is considered to reflect short-term folate intake whereas RBCF is thought to reflect tissue stores and long-term folate status. In this

trial SF and RBCF were positively correlated among the 12 subjects during the self-selected diet. SF and RBCF also were positively correlated among OCA but not among non-OCA separately. It is possible that non-OCA subjects absorb folate more efficiently leading to greater fluctuation in SF levels in response to folate intake.

During the two weeks of high and low folate diet treatments, regardless of the order of treatments, SF variation between subjects decreased progressively. At the end of week 3, after the high and low diets, regardless of diet order, SF varied far less between subjects than it varied during the self-selected diet. This is evidence that variation in SF among individuals is closely related to diet composition as opposed to individual physiological variation.

Several generalizations may be drawn from the findings. Folate intake and resulting SF varied widely between individuals on a self-selected diet. Individual folate intake was consistent over one week and was accurately estimated using a daily Checklist of foods which are good folate sources. SF responded rapidly and drastically to folate restricted and folate supplemented diets. SF response to high and low folate diets was independent of diet order indicating that present folate intake, not previous serum level, is the primary determinant of SF. SF variation between individuals decreased progressively over time on a uniform diet. OCA and non-OCA, the two groups

with the greatest likelihood of differing folate metabolism, responded similarly to folate restricted and folate supplemented diets. Folate intake, rather than metabolic differences, appears to be the primary determinant of an individual's SF concentration. SF changed in response to dietary change in as little as three days which makes it a meaningful index of very short term folate intake. SF has very limited value as a measure of folate status unless accompanied by information about habitual folate intake.

Recommendations for Further Research

The following recommendations for further research are offered:

1. Evaluate the effects of short-term folate restriction and supplementation on a larger sample, representative of a broader population. Wide SF and RBCF variation between individuals makes repeatable results more feasible with a large number of subjects.

2. Folate restriction and supplementation for longer periods of time. Treatment periods of one week were not long enough to establish the plateau in SF and RBCF after an increase or a decrease. Longer treatment periods would allow time for maximum SF and RBCF change.

3. Apply both treatment orders to each subject. Funding limitations did not permit a third treatment period during which subjects would reverse their treatment order. Data for each subject on both diet orders would allow a more precise comparison of effects.

4. Folate restriction and supplementation at different levels. The small sample size in this study did not permit a large variety of treatments. A restricted diet providing more folate and a supplemented diet providing less folate could be used to check the highest dietary folate level which produces a decrease in SF and the lowest level which produces an increase in SF.

5. Supplementation with a food source of folate. Limitations in funding did not permit the additional food folate assays which would have been necessary to use foods as folate supplements. Comparison between SF and RBCF reactions to supplementation with PGA and with food folate would provide information about the effects of different folate forms on blood folate parameters.

6. Evaluation of the Food Folate Checklist on a larger sample for a longer period of time. A single week of

dietary records among a reasonably homogeneous sample did not offer the opportunity to measure individual differences in folate intake over time. The Food Folate Checklist needs to be tested further with a larger number of subjects to establish its validity as an instrument to measure adequacy of folate intake.

Literature Cited

Abad, A.R. and J.F. Gregory. 1985. Effect of diet composition on the bioavailabiliy of radiolabeled mono- and polyglutamyl folates in rats. Fed. Proc. 44:777. (Abstr.).

Anonymous. 1983. "Have the pteroylpolyglutamates a regulatory function?" Nutr. Rev. 41:261-269.

Armstrong, B., R. David, D. Nicol, A. Van Merwyk and D. Larwood, 1974. Hematological, vitamin B_{12}, and folate studies on Seventh-day Adventist vegetarians. Am. J. Clin. Nutr. 27:712-718.

Bailey, L.B., C.S. Mahan, and D. Dimperio. 1980. Folacin and iron status in low-income pregnant adolescents and mature women. Am. J. Clin. Nutr. 33:1997-2001.

Bailey, L.B., P.A. Wagner, G. J. Christakis and C.G. Davis. 1982a. Folacin and iron status of adolescents from low-income rural households Nutr. Res. 2(4): 397-407.

Bailey, L.B., P.A. Wagner, G.J. Chistakis, C.G. Davis, H. Appledorf, P.E. Araujo, E. Dorsey and J.S. Dinning. 1982b. Folacin and iron status and hematological findings in black and Spanish-American adolescents from urban low-income households. Am. J. Clin. Nutr. 35:1023-1032.

Bailey, L.B.,P.A. Wagner, C.G Davis, and J.S. Dinning: Food frequency related to folacin status in adolescents. J. Am. Dietet. Assoc. 84:801, 1984.

Baker, S., and DeMaeyer, E. 1979. Nutritional anemia: its understanding and control with special reference to the work of the World Health Organization. Am. J.Clin. Nutr. 32:368-417.

Baker, S.J., S. Kumar and S.P. Swaminathan. 1965. Excretion of folic acid in bile. The Lancet. March 27.

Banerjee, D.K., A. Maitra, A.K. Basu and J.B. Chatterjea. 1975. Minimal daily requirement of folic acid in normal Indian subjects. Ind. J. Med. Res. 63:45-53.

Bates, C.J., A.E. Black, D.R. Phillips, A.J. Wright and D.A. Southgate. 1982. The discrepancy between folate intakes and the folate RDA. Hum. Nutr: Apl. Nutr. 36A:422-429.

Bills, T., and L. Spatz. 1977. Neutrophilic hypersegmentation as an indicator of incipient folic acid deficiency. Am. J. Clin. Path. 68:263

Breskin, M.W., C.M. Trahms, B. Worthington-Roberts, R.F. Labbe and B. Koslowski. 1985. Supplement use: vitamin intakes and biochemical indexes in 40- to 108-month-old children. J. Am. Dietet. Assoc. 85:49-56.

Brown, J.P., J.M. Scott, F.G. Foster and D.G. Weir. 1973. Ingestion and absorption of naturally occurring pteroylmonoglutamates (folates) in man. Gastroent. 64:223-232.

Burgen, A.S. and N.J. Goldberg. 1962. Absorption of folic acid from the small intestine of the rat. Brit. J. Pharmacol. 19:313-320.

Butterworth, C.E., Jr., C.M. Baugh and C. Krumdieck. 1969. A study of folate absorption and metabolism in man utilizing carbon-14-labeled polyglutamates synthesized by the solid phase method. J. Clin.Invest. 48:1131-1142.

Campillo, B., E. DeGialluly and J. Zittoun. 1985. Supplement of folate - preliminary results from randomized assay. Gastroent. Clin. et Biol. 9:182. (Abstr.).

Chen, M.F., J. W. Hill and P. McIntyre. 1983. The folacin contents of foods as measured by a radiometric microbiologic method. J. Nutr. 113:2192-2196.

Chung, A.S., W.M. Pearson, W.J.Darby, O.N. Miller and G.A. Goldsmith. 1961. Folic acid, vitamin B_6, pantothenic acid and vitamin B_{12} in human dietaries. Am. J. Clin. Nutr. 9:573.

Clark, A.J. and R. Gates. 1983. Folacin status of adolescent females. Fed. Proc. 42:864 (Abstr.).

Colman, N. 1981. "Metabolic role of folate." In Laboratory Assessment of Folate Status: Clinics in Laboratory Medicine. Vol. 4, W.B. Saunders Co., Phila.

Colman, N., E. Barker, M. Barket, R. Green and J. Metz. 1975. Prevention of folate deficiency by food fortification. IV. Identification of target groups in addition to pregnant women in an adult rural population. Am. J. Clin. Nutr. 28:47-476.

Colman, N., N. Hettiarchchy and V. Herbert. 1981. Detection of a milk factor that facilitates folate uptake by intestinal cells. Science. 211:1427-1429.

Cooper, B.A. "Reassessment of folic acid requirement." In Nutrition in Transition: Proceedings of the Western Hemisphere Congress. No. 4. ed. P.L. White and N. Selvey. American Medical Association, 1978.

Daniel, W.A., Jr., E.G. Gaines and D.L. Bennet. 1975. Dietary intakes and plasma concentrations of folate in healthy adolescents. Am. J. Clin. Nutr. 28:363-370.

Daniel, W.H., J.R. Mounger and J. Perkins. 1971. Obstetric and fetal complications in folate deficient adolescent girls. Am. J. Obstet. Gynecol. 111:233-238.

Dong, F.M. and S.M. Oace. 1973. Folate distribution in fruit juices. J. Am. Dietet. Assoc. 62:162-166

Druskin, M. M. Wallen and L. Bonagura. 1962. Anticonvulsant-associated megaloblastic anemia. Response to 25 microgm. of folic acid administered by mouth daily. N. Engl. J. Med. 267:483-485.

Dutram, K. "Serum folate status and dietary folacin intake of adolescent girls." M.S. Thesis, University of Arkansas, 1984.

Edozien, J.C. 1972. National Nutrition Survey, Massachusetts, Report of the Survey Director to the Commissioner for Public Health, Commonwealth of Massachusetts, (Unpublished).

Eichner, E.R., and R.S. Hillman. 1973. Effect of alcohol on serum folate level. J. Clin. Invest. 52:584-591.

Eichner, E., H. Pierce and R. Hillman. 1971. Folate balance in dietary-induced megaloblastic anemia. N. Engl. J. Med. 284:17. Apr. 29.

Ek, J. 1980. Plasma and red cell folate values in newborn infants and their mothers in relation to gestational age. J. Pediatr. 97:288-292.

Elwood, P.C., N.K. Shinton, D.E. Wilson, P. Sweetnam and A.C. Frazer. 1971. Haemoglobin, Vitamin B_{12} and folate levels in the elderly. Br. J. Haematol. 21:557-563.

Ercanli, F., A. Ekpo, E. Carter and J. Lu. 1984. Folacin and Vitamin B_6 status of black adolescent girl. Fed. Proc. 43, Pt.1:1055 (Abstr.).

Georgiou, C.: "Dietary folate and serum folate levels." Ph.D. Thesis, Oklahoma State University, 1986.

Grace, E., S. Emans and D. Drum. 1982. Hematologic abnormalities in adolescents who take oral contraceptive pills. J. Pediatr. 101(5):771-774.

Hages, M. and K. Pietrzik. 1985. Untersuchungen zur Bewertung der Folatversorgung bei Kindern unter Berucksichtigung des colalamin - under Eisenhaushalts. Internat. J. Vit. Nutr. Res. 55:59-67.

Halsted, C.H. "Intestinal absorption and malabsorption of folates." In: Annual Review of Medicine. 31:79-87, 1980.

Hayes, A.N., D.J. Willins and D. Skelton. 1985. Vitamin B_{12} (Cobalamin) and folate blood levels in a geriatric reference group as measured by two kits. Clin. Biochem. 18:56-59.

Health-Aide User's Guide and Reference Manual. Corte Madera; Knossos, Inc., 1983.

Hepner, G.W., C.C. Booth, J. Cowan, A.V. Hoffbrand and D.L. Mollin. 1968. Absorption of crystalline folic acid in man. Lancet. Aug. 10, 1968:302-306.

Herbert, V. 1962a. Experimental nutritional folate deficiency in man. Trans. Assoc. Amer. Phys. 75:307-320.

Herbert, V. "Folic Acid in Human Nutrition." In Proceedings of the Florida Symposium on Micronutrients in Human Nutrition, 1981, pp. 121-138. The Instiute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Herbert, V. 1962b. Minimal daily adult folate requirement. Arch. Int. Med. 110:649-652.

Herbert V. "Summary of the Workshop." In Folic Acid Biochemistry and Physiology in Relation to the Human Nutrition Requirement. Food and Nutrition Board, National Research Council, National Academy of Sciences, Washington, D.C., 1977. pp.277-293.

Herbert, V., N, Colman, M. Spivack, E. Ocasio, V. Ghanta, K. Kimmel, L. Brenner, J. Freundlich and J. Scott. 1975. Folic acid deficiency in the United States: Folate assays in a prenatal clinic. Am. J. Obstet. Gynecol. 123:175-179.

Hillman, R.S. and S.E. Steinberg. 1982. The effects of alcohol on folate metabolism. Ann Rev. of Med. 33:345-354.

Hoffbrand, A.V., E. Tripp and A. Lavoie'. "Folate Polyglutamate Synthesis and Breakdown in Human Cells." In Folic Acid Biochemistry and Physiology in Relation to the Human Nutrition Requirement. Food and Nutrition Board, National Research Council, National Academy of Sciences, Washington, D.C.,1977.

Hoppner, K. 1971. Free and total folate activity in strained baby foods. Can. Inst. Food Sci. Technol. J. 4:51-54.

Hoppner, K., B. Lampi and D.E. Perrin. 1973. Folacin activity of frozen convenience foods. J. Am.Dietet. Assoc. 63:536-539.

Hoppner, K., B. Lampi and D.E. Perrin. 1972. Free and total folate activity in foods available on the Canadian market. J. Can. Inst. Food Sci. Technol. 5:60-66.

Hoppner, K., B. Lampi and D. Smith. "Data on Folacin Activity in Foods: Availability, Applications and Limitations." In Folic Acid: Biochemistry and Physiology in Relation to the Human Nutrition Requirement. National Research Council, National Academy of Sciences. Washington, D.C., 1977.

Hurdle, A.D., D. Barton and I.H. Searles. 1968. A method for measuring folate in food and its application to a hospital diet. Am. J. Clin. Nutr. 21:1202-1207.

Izak, G., M. Rachmilaertz, S. Zan and N. Grossowicz. 1963. The effect of small doses of folic acid in nutritional megaloblastic anemia. Am. J. Clin. Nutr.13:369-377.

Kelly, D., D. Weir, B. Reed and J. Scott. 1979. Effect of anticonvulsant drugs on the rate of folate catabolism in mice. J. Clin. Invest. 64:1089.

Lan, S. J.: "Dietary folacin and serum folacin in adolescent girls." M.S. Thesis, Oklahoma State University, 1982.

Leichter, J., A.F. Landymore and C. Krumdieck. 1979. Folate conjugase activity in fresh vegetables and its effect on the determination of free folate content. Am. J. Clin. Nutr. 32:92-95.

Liebman, M. 1985. Iron and folate status of an adolescent female population. Nutr. Res. 5:621-625.

Lindenbaum, J. 1980. Folate and vitamin B_{12} deficiencies in alcoholism. Seminars in Hematology. 17:119-129.

McCoy, H., M.A. Kenney, A. Kirby, G. Disney, F.G. Ercanli, E. Glover, M. Korslund, H. Lewis, M. Liebman, E. Levant, S. Moak, S. Stallings, T. Wakefield, P. Schilling and S.J. Ritchey. 1984. Nutrient intakes of female adolescents from eight southern states. J. Am. Dietet. Assoc. 84:1453-1460.

McDowell, A., A. Engel, J.T. Massey and K. Maurer. "Plan and Operation of the Second National Health and Nutrition Examination Survey, 1976-80." Vital and Health Statistics, Ser. 1, No. 15, Dept. of HHS Publication No (PHS) 81-1317, U.S. Government Printing Office, Washington, D.C. 1981.

Mitchell, H.K., E.E. Snell and R.J. Williams. 1944. Folic acid I. Concentration from spinach. Am.Chem. Soc. 66:274.

Moscovitch, L.F. and B.A. Cooper. 1973. Folate content of diets in pregnancy: comparison of diets collected at home and diets prepared from dietary records. Am. J. Clin. Nutr. 26:707-714.

National Research Council, National Academy of Sciences.: "Recommended Dietary Allowances," Seventh Revised Edition, 1968. National Academy of Sciences, Washington, D.C., 1968.

National Research Council, National Academy of Sciences.: "Recommended Dietary Allowances," Ninth Revised Edition, 1980. National Academy of Sciences, Washington, D.C., 1980.

Noronha, J.M. and V.S. Aboobaker. 1963. Studies on the folate compounds of human blood. Arch. Biochem Biophys. 101:445-447.

Nutrition Canada. "Nutrition Canada National Survey." Ottawa: Information Canada. 1973.

Olinger, E.J., J.R. Bertino and H.J. Binder. 1973. Intestinal folate absorption. II. Conversion and retention of pteroylmonoglutamate by jejunum. J. Clin. Invest. 52:2138-2145.

Omer, A., N.D. Finlayson, D.J. Shearman, R.R. Samson and R.H. Girdwood. 1970. Plasma and erythrocyte folate in iron deficiency and folate deficiency. Blood. 35:821-828.

Paine, C.J., W.D. Grafton, V.L. Dickson and E.R. Eichner. 1975. Oral contraceptives, serum folate, and hematologic status. J. Am. Med. Assoc. 231:731-733.

Perloff, B.P., and R.B. Butrum, : Folacin in selected foods. J. Am. Dietet. Assoc. 70:161, 1977.

Perry, J. 1971. Folate analogues in normal mixed diets. Br. J. Haematol. 21:435-441.

Perry, J. and I. Chanarin. 1970. Intestinal absorption of reduced folate compounds in man. Br. J. Haematol. 18:329-339.

Perry, J., M. Lamb, M. Laundy, E.H. Reynolds and I. Chanarin. 1976. Role of Vitamin B_{12} in folate coenzyme synthesis. Br. J. Haematol. 32:243-248.

Pietarinen. G., J. Leichter and R. Pratt. 1977. Dietary folate intake and concentration of folate in serum and erythrocytes in women using oral contraceptives. Am. J. Clin. Nutr. 30:375-380.

Poh Tan, S., R.W. Wenlock and D.H. Buss. 1984. Folic acid content of the diet in various types of British household. Hum. Nutr.: Appl. Nutr. and Clin. Prac. 38A:17-22.

Pratt, R.F. and B.A. Cooper. 1971. Folates in plasma and bile of man after feeding folic acid-^{3}H and 5 formyltetrahydrofolate (folinic acid). J. Clin. Invest. 50:455-462.

Pritchard, J.A., D.E. Scott and P.J. Whalley. 1969. Folic acid requirements in pregnancy induced mega-loblastic anemia. J. Am. Med. Assoc. 208:1163-1167.

Prothro, J., M. Mickles and B. Tolbert. 1976. Nutritional status of a population sample in Macon County, Alabama. Am. J. Clin. Ntr. 29:94-104.

Reed, B., D. Weir and J. Scott. 1976. The fate of folate polyglutamates in meat during storage and processing. Am. J. Clin. Nutr. 29:1393

Retief, F.P. and D. Phil. 1969. Urinary folate excretion after ingestion of pteroylmonoglutamic acid and food folate. Am. J. Clin. Ntr. 22:352-355.

Rhode, B.M., B.A. Cooper and F.A. Farmer. 1983. Effect of orange juice, folic acid, and oral contraceptives on serum folate in women taking a folate-restricted diet. J. Am. Coll. Nutr. 2:221-230.

Riester, P.T. and C.I. Waslien. 1975. Folacin status of nine year old girls in Alabama. Fed. Proc. 34:904. (Abstr.).

Rodriguez, M.S. 1978. A conspectus of research on folacin requirements of man. J. Nutr. 108:1983-2103.

Rosenberg, I.H., 1977. "Role of Intestinal Conjugase in the Control of the Absorption of Polyglutamyl Folates." in Folic Acid: Biochemistry and Physiology in Relation to the Human Nutrition Requirement. National Academy of Sciences. Washington, D.C.

Rosenberg, I.H., B.B. Bowman, B.A. Cooper, C.H. Halsted and J. Ludenbaum. 1982. Folate nutrition in the elderly. Am. J. Clin. Nutr. 36:1060-1066.

Rosenberg, I.H. and H.A. Godwin. 1971. The digestion and absorption of dietary folate. Gastroent. 60:445-463.

Rosenberg, I.H., R.R. Streiff, H.A. Godwin and W.B. Castle. 1969. Absorption of polyglutamic folate: participation of deconjugating enzymes of the intestinal mucosa. New Eng. J. Med. 280:985.

Russell, R.M., I.H. Rosenberg, P.D. Wilson, F.L. Iber, E.B. Oaks, A.C. Giovetti, C.L. Otradovec, P.A. Karwoski and A.W. Press. 1983. Increased urinary excretion and prolonged turnover time of folic acid during ethanol ingestion. Am. J. Clin. Ntr. 38:64-70.

Santini, R., Jr., F.M. Berger, G. Berdasco, T.W. Sheehy, J.A. Aviles and I. Davila. 1962. Folic acid activity in Puerto Rican foods. J. Am. Dietet. Assoc. 41:562-567.

Santini, R. and J.J. Corcino. 1974. Analysis of some nutrients of the Puerto Rican diet. Am. J. Clin. Ntr. 27:840-844.

Sauberlich, H.E. 1977. "Detection of Folic Acid Deficiency in Populations." in Folic Acid: Biochemistry and Physiology in Relation to the Human Nutrition Requirement. National Academy of Sciences, Washington, D.C., 1977.

Scott, J.M., V. Ghanta and V. Herbert. Trouble-free microbiologic serum and red cell folate assays. 1974. Am. J. Med. Tech. 40:125-134.

Sempos, C.T., N.E. Johnson, E.L. Smith and C. Gilligan. 1984. A two-year dietary survey of middle-aged women: repeated dietary records as a measure of usual intake. J. Am. Dietet. Assoc. 84:1008-1013.

Senti, F., and S. Pilch, editors. 1985. Analysis of folate data from the Second National Health and Nutrition Examination Survey (NHANES II). J. Nutr. 115:1398-1402.

Senti,F.R. and S.M. Pilch, Editors. Assessment of the Folate Nutritional Status of the U.S. Population Based on Data Collected in the Second National Health and Nutrition Examination Survey, 1976-1980. Federation of American Societies for Experimental Biology. Bethesda, Maryland, 1984.

Shevchuk, O. and D.A. Roe. 1985. Effects of aspirin ingestion upon blood and urine folate concentration and plasma folate binding. 44:1858. (Abstr.).

Shin, Y.S., K.U. Buehring and E.L. Stokstad. 1974. Studies of folate compounds in nature: Folate compounds in rat kidney and red blood cells. Arch. Biochem. Biophys. 163:211-224.

Shojania, A.M. and G. Hornady. 1969. Effect of anti-microbial agents on serum folate assay. Am. J. Clin. Path. 52:454-456.

Spring, J.A., J. Robertson and D.H. Buss. 1979. Trace nutrients. Magnesium, copper, zinc, vitamin B_6, vitamin B_{12} and folic acid in the British house-hold food supply. Br. J. Nutr. 41:487-493.

Steel, R.G.D. and J.H. Torrie,: Principles and Procedures of Statistics: A Biometrical Approach. Second Edition. New York: McGraw-Hill Book Company, 1980.

Steinberg, S.E., C.L. Campbell and R.S. Hillman. 1979. Kinetics of the normal folate enterohepatic cycle. J. Clin Invest. 64:83-88.

Stokstad, E.L., M.M. Chan and J.E. Watson. "The metabolic relationship between folic acid, Vitamin B_{12}, methionine and thyroxine." In Proceedings of the Florida Symposium on Micronutrients in Human Nutrition, 1981. The Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Stokstad, E.L., Y.S. Shin and T.Tamura. "Distribution of Folate forms in Food and Folate Availability." In Folic Acid: Biochemistry and Physiology in Relation to the Human Nutrition Requirement. Food and Nutrition Board, National Academy of Sciences, Washington, D.C., 1977.

Tamura, T. and E.L. Stokstad. 1973. The availability of food folate in man. Br. J. Haematol. 25:513-532.

Ten-State Nutrition Survey, 1968-1970. Part III-Clinical, Anthropometry, Dental. U.S. Department of Health, Education and Welfare, Health Services and Mental Health Administration Center for Disease Control, Atlanta, Georgia. 1972.

Thenen, S.W. 1982. Folacin content of supplemental food for pregnancy. J. Am. Dietet. Assoc. 80:237-241.

Toepfer, E., E. Zook, M. Orr and L. Richardson. "Folic acid Content of Foods.: U.S.D.A. Agriculture Handbook #29." Washington, D.C., 1951.

Tsui, J., J. Nordstrom and M. Kohrs. 1985. Effect of folic acid supplmentation in adolescents. Fed Proc. 43, Pt. 1:986. (Abstr.).

Tyerman, M.J., J.E. Watson, B. Shane, D.E. Schutz and E.L. Stokstad. 1977. Identification of glutamate chain lengths of endogenous folylpoly - gamma-glutamates in rat tissues. Biochim. et Biophys. Acta. 497:234-240.

Van de Mark, M.S. and A. C. Wright. 1972. Hemoglobin and folate levels of pregnant teenagers. J. Am. Dietet. Assoc. 61:511-516.

Wagner, P., L. Bailey, G. Christakis, C. Davis, H. Appledorf and J. Dinning. 1981. "Folacin and Iron Status and Hematological Findings in an Elderly Population." In Proceedings of the Florida Symposium on Micronutrients in Human Nutrition. 1981. The Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Webster, L. and J. Leeming. 1979. Erythrocyte folate levels in young and old. J. Am. Geriat. Soc. 27:451-454.

Whitehead, V.M. 1973. Polygammaglutamyl metabolites of folic acid in human liver. The Lancet. April 7, 1973: 743-745.

Whitehead, V.M. and B.A. Cooper. 1967. Absorption of unaltered folic acid from the gastro-intestinal tract in man. Br. J. Haematol. 13:679-686.

World Health Organization. Nutritional Anemias: Report of WHO group of Experts. Technical Report Series #503. World Health Organization, Geneva, 1972.

Yadrick, K.: "The effects of supplementation with zinc or zinc and iron on zinc, iron and copper status." Ph.D. Thesis, Oklahoma State University, 1986.

APPENDIXES

APPENDIX A

24-HOUR DIETARY RECORD

DAILY FOOD LIST

Name_____

Date_____

Subject No._____

Daily Food Record

Time of day	Food	Amount Eaten	Where Eaten

CG 6/14/85

Daily Food List	FRIDAY 7/12	SUNDAY 7/14	TUESDAY 7/16	THURSDAY 7/18	SATURDAY 7/20	MONDAY 7/22	WEDNESDAY 7/24
Breakfast							
Grape Juice - 4 oz.							
Cheerios - 1 oz.							
Milk - 4 oz.							
Supplements: Multivitamin/mineral							
Experimental supplement							
Lunch							
American Cheese - 1 oz.							
Turkey - 2 oz.							
Rye Bread - 1 slice							
Applesauce - 3/4 cup							
Fig Bars - 2							
Dinner							
Sirloin Steak - 4 oz.							
Saltines - 10							
Peaches in Light Syrup - 1/2 cup							
Vanilla Ice Cream - 1/3 cup							
Miscellaneous (provided by the study)							
Margarine pats							
Jelly packets							
Mayonnaise packets							
Mustard packets							
Catsup packets							
Half and Half packets							
Life Savers							
Metamucil packets							
Miscellaneous (supplied by participant)							
Coffee - regular or decaffeinated							
Tea - regular not herbal							
Sugar - white only							
Soft drinks - Coke, Pepsi, Dr. Pepper, 7-Up (Regular or diet)							
Sour Balls, Jelly Beans, Velamints							

CG 6/25/85

Daily Food List	SATURDAY 7/13	MONDAY 7/15	WEDNESDAY 7/17	FRIDAY 7/19	SUNDAY 7/21	TUESDAY 7/23	THURSDAY 7/25
Breakfast							
Apple Juice - 6 oz.							
100 % Natural Cereal - 1 oz.							
Milk - 4 oz.							
Supplements: Multivitamin-mineral							
Experimental Supplement							
Lunch							
Swiss Cheese - 2 oz.							
Ham - 1 oz.							
Rye Bread - 1 slice							
Pineapple chunks/natural juice - 1/2 cup							
Hershey Bar - 1 oz.							
Dinner							
Chicken Breast - 3 oz.							
Saltines - 10							
Purple Plums in syrup - 3/4 cup							
Popsicle - 1							
Miscellaneous (provided by the study)							
Margarine pats							
Jelly packets							
Mayonnaise packets							
Mustard packets							
Catsup packets							
Half and Half packets							
Life Savers							
Metamucil packets							
Miscellaneous (supplied by participant)							
Coffee - regular or decaffeinated							
Tea - regular not herbal							
Sugar - white only							
Soft drinks - Coke, Pepsi, Dr. Pepper, 7-Up (Regular or diet)							
Sour Balls, Jelly Beans Velamints							

CG/6/85

APPENDIX B

ANALYSIS OF EXPERIMENTAL DIET

HAZLETON LABORATORIES AMERICA, INC. Chemical & BioMedical Sciences Division
3301 KINSMAN BLVD. • P.O. BOX 7545 • MADISON, WISCONSIN 53707 • PHONE (608) 241-4471 • TLX 703956 HAZRAL MDS UD

CORRECTED REPORT OF ANALYSIS

CONNIE GEORGIOU
OKLAHOMA STATE UNIVERSITY
DEPARTMENT FNTA, HEW
ROOM 425
STILLWATER, OK 74078

SAMPLE NUMBER: 50708022
DATE ENTERED: 07/25/86
REPORT PRINTED: 08/19/86

FOOD COMPOSITE: 2 DAY MENU

PURCHASE ORDER NUMBER: R21076 & R233452

ASSAY	ANALYSIS	UNITS
PROTEIN (N X 6.25)	5.5	G/100 G
MOISTURE, 70 DEGREE VAC. OVEN	64.4	G/100 G
FAT	5.9	G/100 G
ASH	1.0	G/100 G
CRUDE FIBER	.8	G/100 G
CARBOHYDRATES	22.4	G/100 G
CALORIES	165.	CALORIES/100 G
FOLIC ACID	.078	MCG/G
FOLIC ACID	.077	MCG/G

TOTAL DIET WEIGHT

WET WEIGHT (AS RECEIVED)	1899	GRAMS

APPENDIX C

CONSENT FORM APPLICATION

FOR PARTICIPATION

INSTRUCTION SHEET

Informed Consent for Participation
In Folic Acid Study

Dear _____.

Thank you for volunteering to participate in a three-week nutrition research study (July 5 - July 25, 1985) to provide information about the assessment of folic acid status in women. Please read the following agreement carefully and sign it indicating your informed consent to participate in the study:

AGREEMENT

During the first week of the study I can eat or drink whatever I wish except vitamin or mineral supplements. During that week I will keep a written record of everything I eat and drink. For the second and third weeks of the study I will eat and drink only the foods and supplements provided to me by the project leader. On three mornings each week (Monday, Wednesday and Friday) I will come to the OSU Student Hospital lab and have a very small amount (1-2 teaspoons) of blood drawn by venipuncture. I understand that I cannot eat or drink anything except water from midnight the night before,until my blood is drawn between 8:00 and 8:30 A.M. on these mornings. I will be provided with breakfast on campus these mornings if I wish.

I am aware that the diet I will be given provides 100% of the Recommended amounts of all essential nutrients except folic acid which will be provided at not less than the adult minimum daily requirement. All of the dietary and health information I provide will be held in strictest confidence. I understand that I will be treated with every consideration for my health and comfort during the study and that there is no reason to expect any side effects from the experimental treatments. I also understand that I can withdraw from the study at any time if I wish.

_____ _____
Participant's signature Date

_____ _____
Project Leader's signature Date

Application for Participation in Folic Acid Study

Name_____ Today's Date_____

Height_____ Your Birthdate_____

Weight_____

How much has your weight fluctuated in the last six months? _____

Have you ever had diabetes_____ Heart disease_____

anemia_____ High blood pressure_____

Other serious illness or condition of poor health_____

Are you allergic to any foods_____ or any drugs _____

If so, what are they?_____

Are you Pregnant_____ taking oral contraceptives_____

If yes, what brand_____?

Are you taking vitamin or mineral supplements_____?

If yes, what kind, brand and amount do you take daily?
(Example: Naturemade Vitamin C, one 250 mg tablet daily)

_____

_____

Are you taking any medications on a regular basis _____? If so, what are they_____and how often do you take them_____?

Do you drink alcoholic beverages_____? If yes, what type_____ and what is your average weekly intake_____?

What was the beginning date of your last menstrual period_____ your usual length of period_____and usual duration of your menstrual cycle_____?

What is your usual pattern of exercise? Describe briefly the types of exercise you engage in on a regular basis, how often you do them, and how long each time.
(Example: Jogging, 3 times/week, 30 minutes)

_____

_____

Please return form to Connie Georgiou, HEW 407, Extension 5039

CG 6/14/85

INSTRUCTIONS TO PARTICIPANTS IN
FOLIC ACID STUDY

WEEK 1 - Friday, July 5 - Thursday, July 11

During this week you are free to eat and drink whatever you choose except that you CANNOT TAKE ANY VITAMIN OR MINERAL SUPPLEMENTS.

Daily Food Record

Each day you will record all food and drink (except water) consumed on the Daily Food Record forms which will be given to you. It is easiest to record each food eaten as soon as possible. Record amounts in whatever units are convenient. Note weights of unit packages eaten, for example: (3/4 oz. Hershey milk chocolate bar, 12 oz. can of orange crush, 20 g. bag of potato chips, one of 18 slices of bread in a 1-pound loaf). Use brand names when possible (1 Big Mac, 3 Hostess twinkies, 1/2 cup Cheerios). You will be given a measuring cup to measure foods such as cereals, fruits, vegetables, rice, salads and all drinks. Try to remember to measure all food and drinks in this cup.

Note whether a food is eaten raw or cooked and the method of preparation, for example: (1 large egg, scrambled in margarine). Be sure to include sugar in tea, butter on vegetables, gravy on potatoes, etc. Note the type of bread you eat and its brand name, for example: (Mrs. Wright's Cracked Wheat). Note where each food was eaten.

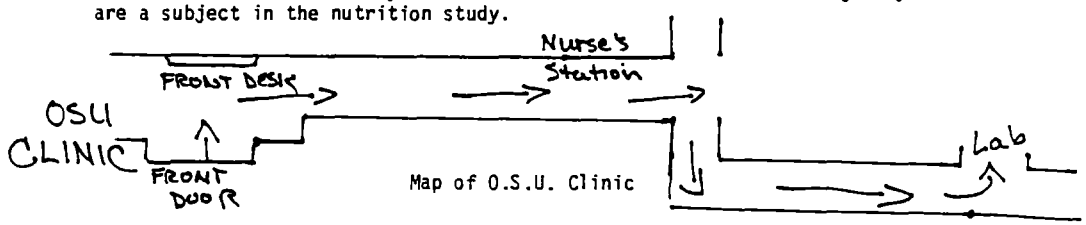
Daily Food Checklist

Each day, at the end of the day, also fill out a Daily Food Checklist indicating how often and how much you ate of each food listed. This is to be filled out IN ADDITION TO the Daily Food Record.

Blood Collection

On Monday, Wednesday and Friday, July 8, 10 and 12 blood will be drawn from all participants. Please come to the O.S.U. Hospital lab between 8:00 and 8:30 A.M. on those dates. In preparation for having blood drawn, DO NOT EAT OR DRINK ANYTHING, except water, after midnight the night before blood is to be drawn. Do not have breakfast before blood is drawn. If you wish you will be given breakfast in the Nutrition lab (HEW407) afterwards.

When you go to the O.S.U. hospital you need not stop at the front desk of the clinic. Go directly to the Lab. Tell the medical technologist you are a subject in the nutrition study.



Map of O.S.U. Clinic

In the unusual event that you find you will unexpectedly be late or unable to come to the lab at the specified time please contact me by telephone at home (743-0955) before 7:45 A.M. or at the O.S.U. Clinic Lab (624-7514) between 8:00 and 8:30 A.M.. Each blood sample is essential to the study and it is very important that none be missed.

Please do not donate blood during the study.

WEEKS 2 AND 3 - Friday, July 12 - Friday morning, July 26

During these weeks you will be eating the experimental diet.

The Experimental Diet

During weeks 2 and 3 all of the food and supplements you will eat will be provided to you to take home, prepare and eat. THIS IS THE ONLY FOOD YOU MAY EAT DURING THESE WEEKS with the exception of the following items which you may have in unlimited amounts:

Coffee - regular or decaffeinated
Tea - regular not herbal
Diet or regular Coke, Pepsi, Dr. Pepper and 7-Up

(White sugar and/or milk or cream provided in the diet are the only things which may be added to drinks)

Life savers
Jelly beans
Sour balls - any flavor (These are hard candy balls)
Velamints
Wrigley's spearmint or doublemint gum
Trident gum - any flavor
Carefree gum - any non-fruit flavor
Bubble Yum bubble gum - original flavor only - sugar free or regular

The diet you receive will be individually calculated to meet your calorie needs without gaining or losing weight. It is absolutely necessary that you eat all of each day's food and supplements on that day. You may eat any individual food at any time during the day but by the end of each day all foods for that day should have been eaten.

You will be given a multi-vitamin/mineral supplement to take every other day during the experimental diet. You will also have one type of supplement to take daily during the first week of the experimental diet and a different supplement to take daily during the second week. You may be taking a different supplement than somebody else in the study. All supplements should be taken with breakfast. That means AFTER BLOOD COLLECTION, not before.

You will have a checklist for each day to mark off all the foods eaten. Please fill this out every day.

The last day of the experimental diet will be Thursday, July, 25, but the last blood collection isn't until Friday, July 26.

Blood Collection

On Monday, Wednesday and Friday, July 15, 17, 19, 22, 24 and 26 blood will be drawn from all participants at the O.S.U. Hospital Lab according to the same procedure as during week 1.

After your blood is drawn Friday, July 26 your participation in the study will be completed.

If you have questions at any time before or during the study please feel free to call me at home (743-0955) or at the Nutrition lab (624-5039). Study results will be made available to all participants.

Thank you very much for your assistance and cooperation in this research project. I look forward to working with you over the next few weeks.

Connie Georgiou
HEW 407

CG 6/14/85

APPENDIX D

DATA FOR INDIVIDUAL SUBJECTS

OBS	GROUP	STUDENT	DAY	DI	FOLATE	VITC	VITA	FOODFREQ	SF	RBCF	HCT	WT	TML	OCA	STU	DT	DX	ORDER
1	A	2A	1	SS	196	22	637	45.0	.	.	.	.	T	A	2	SS	1	LH
2	B	2B	1	SS	175	128	895	35.0	.	.	.	.	T	B	2	SS	1	LH
3	A	1A	1	SS	167	95	881	37.5	.	.	.	.	T	A	1	SS	1	HL
4	B	1B	1	SS	184	106	1309	20.0	.	.	.	.	T	B	1	SS	1	HL
5	A	3A	1	SS	109	119	524	25.0	.	.	.	.	M	A	3	SS	1	HL
6	B	3B	1	SS	56	52	949	30.0	.	.	.	.	M	B	3	SS	1	HL
7	A	4A	1	SS	128	40	720	25.0	.	.	.	.	M	A	4	SS	1	LH
8	B	4B	1	SS	211	114	990	50.0	.	.	.	.	M	B	4	SS	1	LH
9	A	5A	1	SS	50	36	233	17.5	.	.	.	.	L	A	5	SS	1	HL
10	B	5B	1	SS	70	95	1222	17.5	.	.	.	.	L	B	5	SS	1	HL
11	A	6A	1	SS	80	128	818	5.0	.	.	.	.	L	A	6	SS	1	LH
12	B	6B	1	SS	169	111	1517	30.0	.	.	.	.	L	B	6	SS	1	LH
13	A	1A	2	SS	311	141	126	55.0	.	.	.	.	T	A	1	SS	2	HL
14	B	1B	2	SS	157	164	1648	25.0	.	.	.	.	T	B	1	SS	2	HL
15	A	2A	2	SS	97	16	342	30.0	.	.	.	.	T	A	2	SS	2	LH
16	B	2B	2	SS	197	42	449	17.5	.	.	.	.	T	B	2	SS	2	LH
17	A	3A	2	SS	106	99	695	27.5	.	.	.	.	M	A	3	SS	2	HL
18	B	3B	2	SS	125	133	905	27.5	.	.	.	.	M	B	3	SS	2	HL
19	A	4A	2	SS	150	125	1153	20.0	.	.	.	.	M	A	4	SS	2	LH
20	B	4B	2	SS	235	117	869	40.0	.	.	.	.	M	B	4	SS	2	LH
21	A	5A	2	SS	54	128	2650	10.0	.	.	.	.	L	A	5	SS	2	HL
22	B	5B	2	SS	81	49	844	10.0	.	.	.	.	L	B	5	SS	2	HL
23	A	6A	2	SS	81	62	401	22.5	.	.	.	.	L	A	6	SS	2	LH
24	B	6B	2	SS	111	62	521	30.0	.	.	.	.	L	B	6	SS	2	LH
25	A	1A	3	SS	239	132	1294	25.0	.	.	.	.	T	A	1	SS	3	HL
26	B	1B	3	SS	156	145	1493	20.0	.	.	.	.	T	B	1	SS	3	HL
27	A	2A	3	SS	97	136	384	30.0	.	.	.	.	T	A	2	SS	3	LH
28	B	2B	3	SS	185	115	313	70.0	.	.	.	.	T	B	2	SS	3	LH
29	A	3A	3	SS	258	128	1413	47.5	.	.	.	.	M	A	3	SS	3	HL
30	B	3B	3	SS	116	32	1108	30.0	.	.	.	.	M	B	3	SS	3	HL
31	A	4A	3	SS	272	338	2400	47.5	.	.	.	.	M	A	4	SS	3	LH
32	B	4B	3	SS	307	125	949	95.0	.	.	.	.	M	B	4	SS	3	LH
33	A	5A	3	SS	101	24	431	37.5	.	.	.	.	L	A	5	SS	3	HL
34	B	5B	3	SS	129	38	240	7.5	.	.	.	.	L	B	5	SS	3	HL
35	A	6A	3	SS	81	32	1825	15.0	.	.	.	.	L	A	6	SS	3	LH
36	B	6B	3	SS	152	79	370	30.0	.	.	.	.	L	B	6	SS	3	LH
37	A	1A	4	SS	187	169	1061	40.0	23.0	.	.	.	T	A	1	SS	4	HL
38	B	1B	4	SS	141	186	1509	30.0	23.5	.	.	.	T	B	1	SS	4	HL
39	A	2A	4	SS	85	8	260	35.0	13.9	.	.	.	T	A	2	SS	4	LH
40	B	2B	4	SS	110	4	127	50.0	9.4	.	.	.	T	B	2	SS	4	LH
41	A	3A	4	SS	135	111	1232	30.0	9.2	.	.	.	M	A	3	SS	4	HL
42	B	3B	4	SS	245	127	1228	65.0	10.2	.	.	.	M	B	3	SS	4	HL
43	A	4A	4	SS	230	148	1878	7.5	23.5	.	.	.	M	A	4	SS	4	LH
44	B	4B	4	SS	369	151	1741	55.0	7.7	.	.	.	M	B	4	SS	4	LH
45	A	5A	4	SS	250	37	757	32.5	4.8	.	.	.	L	A	5	SS	4	HL
46	B	5B	4	SS	24	63	405	0.0	2.3	.	.	.	L	B	5	SS	4	HL
47	A	6A	4	SS	41	32	151	10.0	.	.	.	.	L	A	6	SS	4	LH
48	B	6B	4	SS	259	96	917	50.0	8.6	.	.	.	L	B	6	SS	4	LH
49	A	1A	5	SS	254	134	1327	45.0	.	.	.	.	T	A	1	SS	5	HL
50	B	1B	5	SS	185	145	1148	30.0	.	.	.	.	T	B	1	SS	5	HL
51	A	2A	5	SS	76	43	1236	15.0	.	.	.	.	T	A	2	SS	5	LH
52	B	2B	5	SS	47	6	174	10.0	.	.	.	.	T	B	2	SS	5	LH
53	A	3A	5	SS	356	168	2815	80.0	.	.	.	.	M	A	3	SS	5	HL
54	B	3B	5	SS	121	73	2164	28.0	.	.	.	.	M	B	3	SS	5	HL

OBS	GROUP	STUDENT	DAY	DI	FOLATE	VITC	VITA	FOODFREQ	SF	RBCF	HCT	WT	TML	OCA	STU	DT	DX	ORDER
55	A	4A	5	SS	217	232	1900	12.5	.	.	.	.	M	A	4	SS	5	LH
56	B	4B	5	SS	234	141	1150	75.0	.	.	.	.	M	B	4	SS	5	LH
57	A	5A	5	SS	191	178	2031	25.0	.	.	.	.	L	A	5	SS	5	HL
58	B	5B	5	SS	23	37	169	0.0	.	.	.	.	L	B	5	SS	5	HL
59	A	6A	5	SS	83	19	276	12.5	.	.	.	.	L	A	6	SS	5	LH
60	B	6B	5	SS	146	98	362	40.0	.	.	.	.	L	B	6	SS	5	LH
61	A	1A	6	SS	93	49	681	25.0	26.3	.	.	.	T	A	1	SS	6	HL
62	B	1B	6	SS	106	103	879	5.0	12.5	.	.	.	T	B	1	SS	6	HL
63	A	2A	6	SS	147	23	680	5.0	13.8	.	.	.	T	A	2	SS	6	LH
64	B	2B	6	SS	242	132	545	35.0	10.0	.	.	.	M	B	2	SS	6	LH
65	A	3A	6	SS	183	253	765	35.0	7.2	.	.	.	M	A	3	SS	6	HL
66	B	3B	6	SS	74	28	1350	17.5	4.8	.	.	.	M	B	3	SS	6	HL
67	A	4A	6	SS	119	97	566	30.0	22.6	.	.	.	M	A	4	SS	6	LH
68	B	4B	6	SS	103	18	732	35.0	7.3	.	.	.	M	B	4	SS	6	LH
69	A	5A	6	SS	56	47	458	5.0	6.5	.	.	.	L	A	5	SS	6	HL
70	B	5B	6	SS	161	61	871	12.5	2.1	.	.	.	L	B	5	SS	6	HL
71	A	6A	6	SS	101	19	575	20.0	.	.	.	.	L	A	6	SS	6	LH
72	B	6B	6	SS	143	86	674	45.0	8.6	.	.	.	L	B	6	SS	6	LH
73	A	1A	7	SS	304	210	1584	45.0	.	.	.	.	T	A	1	SS	7	HL
74	B	1B	7	SS	145	223	1642	20.0	.	.	.	.	T	B	1	SS	7	HL
75	A	2A	7	SS	90	11	188	30.0	.	.	.	.	T	A	2	SS	7	LH
76	B	2B	7	SS	120	71	1258	25.0	.	.	.	.	T	B	2	SS	7	LH
77	A	3A	7	SS	335	94	982	92.5	.	.	.	.	M	A	3	SS	7	HL
78	B	3B	7	SS	45	14	676	2.5	.	.	.	.	M	B	3	SS	7	HL
79	A	4A	7	SS	58	93	860	17.5	.	.	.	.	M	A	4	SS	7	LH
80	B	4B	7	SS	537	359	1393	75.0	.	.	.	.	M	B	4	SS	7	LH
81	A	5A	7	SS	71	33	391	0.0	.	.	.	.	L	A	5	SS	7	HL
82	B	5B	7	SS	80	95	254	10.0	.	.	.	.	L	B	5	SS	7	HL
83	A	6A	7	SS	61	37	518	25.0	.	.	.	.	L	A	6	SS	7	LH
84	B	6B	7	SS	233	112	2690	40.0	.	.	.	.	L	B	6	SS	7	LH
85	A	1A	8	SS	.	.	.	.	17.7	362.9	41.0	104	T	A	1	SS	8	HL
86	B	1B	8	SS	.	.	.	.	21.9	394.9	46.0	110	T	B	1	SS	8	HL
87	A	2A	8	SS	.	.	.	.	12.6	230.8	43.0	.	T	A	2	SS	8	LH
88	B	2B	8	SS	.	.	.	.	8.1	193.6	39.0	126	T	B	2	SS	8	LH
89	A	3A	8	SS	.	.	.	.	8.1	380.5	50.0	113	M	A	3	SS	8	HL
90	B	3B	8	SS	.	.	.	.	6.2	248.4	41.5	.	M	B	3	SS	8	HL
91	A	4A	8	SS	.	.	.	.	18.2	338.8	39.0	.	M	A	4	SS	8	LH
92	B	4B	8	SS	.	.	.	.	7.4	188.3	40.0	.	M	B	4	SS	8	LH
93	A	5A	8	SS	.	.	.	.	5.3	258.1	43.0	.	L	A	5	SS	8	HL
94	B	5B	8	SS	.	.	.	.	3.2	29.1	41.5	.	L	B	5	SS	8	HL
95	A	6A	8	SS	.	.	.	.	4.3	188.0	42.0	.	L	A	6	SS	8	LH
96	B	6B	8	SS	.	.	.	.	8.3	190.3	41.0	.	L	B	6	SS	8	LH
97	A	1A	11	HI	.	.	.	.	21.4	.	.	.	T	A	1	HI	11	HL
98	B	1B	11	HI	.	.	.	.	13.2	.	.	.	T	B	1	HI	11	HL
99	A	2A	11	LO	.	.	.	.	10.8	.	.	.	T	A	2	LO	11	LH
100	B	2B	11	LO	.	.	.	.	6.5	.	.	.	T	B	2	LO	11	LH
101	A	3A	11	HI	.	.	.	.	7.5	.	.	.	M	A	3	HI	11	HL
102	B	3B	11	HI	.	.	.	.	6.9	.	.	.	M	B	3	HI	11	HL
103	A	4A	11	LO	.	.	.	.	15.8	.	.	.	M	A	4	LO	11	LH
104	B	4B	11	LO	.	.	.	.	7.2	.	.	.	M	B	4	LO	11	LH
105	A	5A	11	HI	.	.	.	.	6.7	.	.	.	L	A	5	HI	11	HL
106	B	5B	11	HI	.	.	.	.	6.4	.	.	.	L	B	5	HI	11	HL
107	A	6A	11	LO	.	.	.	.	6.9	.	.	.	L	A	6	LO	11	LH
108	B	6B	11	LO	.	.	.	.	5.7	.	.	.	L	B	6	LO	11	LH

OBS	GROUP	STUDENT	DAY	DI	FOLATE	VITC	VITA	FOODFREQ	SF	RBCF	HCT	WT	TML	OCA	STU	DT	DX	ORDER
109	A	1A	13	HI	.	.	.	.	22.6	.	.	.	T	A	1	HI	13	HL
110	B	1B	13	HI	.	.	.	.	19.5	.	.	.	T	B	1	HI	13	HL
111	A	2A	13	LO	.	.	.	.	9.3	.	.	.	T	A	2	LO	13	LH
112	B	2B	13	LO	.	.	.	.	7.2	.	.	.	T	B	2	LO	13	LH
113	A	3A	13	HI	.	.	.	.	10.5	.	.	.	M	A	3	HI	13	HL
114	B	3B	13	HI	.	.	.	.	8.7	.	.	.	M	B	3	HI	13	HL
115	A	4A	13	LO	.	.	.	.	18.1	.	.	.	M	A	4	LO	13	LH
116	B	4B	13	LO	.	.	.	.	7.5	.	.	.	M	B	4	LO	13	LH
117	A	5A	13	HI	.	.	.	.	5.7	.	.	.	L	A	5	HI	13	HL
118	B	5B	13	HI	.	.	.	.	5.6	.	.	.	L	B	5	HI	13	HL
119	A	6A	13	LO	.	.	.	.	4.6	.	.	.	L	A	6	LO	13	LH
120	B	6B	13	LO	.	.	.	.	7.6	.	.	.	L	B	6	LO	13	LH
121	A	1A	15	HI	.	.	.	.	27.0	396.5	41.0	104	T	A	1	HI	15	HL
122	B	1B	15	HI	.	.	.	.	16.7	376.3	45.0	110	T	B	1	HI	15	HL
123	A	2A	15	LO	.	.	.	.	9.3	211.3	41.0	139	T	A	2	LO	15	LH
124	B	2B	15	LO	.	.	.	.	6.4	169.3	42.0	126	T	B	2	LO	15	LH
125	A	3A	15	HI	.	.	.	.	11.2	285.0	48.0	113	M	A	3	HI	15	HL
126	B	3B	15	HI	.	.	.	.	11.2	251.2	42.0	146	M	B	3	HI	15	HL
127	A	4A	15	LO	.	.	.	.	12.4	268.1	40.0	153	M	A	4	LO	15	LH
128	B	4B	15	LO	.	.	.	.	5.8	198.4	38.5	176	M	B	4	LO	15	LH
129	A	5A	15	HI	.	.	.	.	9.1	217.9	39.5	124	L	A	5	HI	15	HL
130	B	5B	15	HI	.	.	.	.	6.1	59.6	42.0	151	L	B	5	HI	15	HL
131	A	6A	15	LO	.	.	.	.	2.8	125.9	39.0	116	L	A	6	LO	15	LH
132	B	6B	15	LO	.	.	.	.	6.4	177.7	41.0	113	L	B	6	LO	15	LH
133	A	1A	18	LO	.	.	.	.	18.1	.	.	.	T	A	1	LO	3	HL
134	B	1B	18	LO	.	.	.	.	15.2	.	.	.	T	B	1	LO	3	HL
135	A	2A	18	HI	.	.	.	.	12.2	.	.	.	T	A	2	HI	3	LH
136	B	2B	18	HI	.	.	.	.	9.6	.	.	.	T	B	2	HI	3	LH
137	A	3A	18	LO	.	.	.	.	6.3	.	.	.	M	A	3	LO	3	HL
138	B	3B	18	LO	.	.	.	.	9.8	.	.	.	M	B	3	LO	3	HL
139	A	4A	18	HI	.	.	.	.	16.6	.	.	.	M	A	4	HI	3	LH
140	B	4B	18	HI	.	.	.	.	9.7	.	.	.	M	B	4	HI	3	LH
141	A	5A	18	LO	.	.	.	.	8.6	.	.	.	L	A	5	LO	3	HL
142	B	5B	18	LO	.	.	.	.	5.5	.	.	.	L	B	5	LO	3	HL
143	A	6A	18	HI	.	.	.	.	5.2	.	.	.	L	A	6	HI	3	LH
144	B	6B	18	HI	.	.	.	.	7.8	.	.	.	L	B	6	HI	3	LH
145	A	1A	20	LO	.	.	.	.	14.0	.	.	.	T	A	1	LO	5	HL
146	B	1B	20	LO	.	.	.	.	11.1	.	.	.	T	B	1	LO	5	HL
147	A	2A	20	HI	.	.	.	.	12.8	.	.	.	T	A	2	HI	5	LH
148	B	2B	20	HI	.	.	.	.	9.8	.	.	.	T	B	2	HI	5	LH
149	A	3A	20	LO	.	.	.	.	9.2	.	.	.	M	A	3	LO	5	HL
150	B	3B	20	LO	.	.	.	.	8.1	.	.	.	M	B	3	LO	5	HL
151	A	4A	20	HI	.	.	.	.	18.3	.	.	.	M	A	4	HI	5	LH
152	B	4B	20	HI	.	.	.	.	7.6	.	.	.	M	B	4	HI	5	LH
153	A	5A	20	LO	.	.	.	.	5.5	.	.	.	L	A	5	LO	5	HL
154	B	5B	20	LO	.	.	.	.	4.6	.	.	.	L	B	5	LO	5	HL
155	A	6A	20	HI	.	.	.	.	5.3	.	.	.	L	A	6	HI	5	LH
156	B	6B	20	HI	.	.	.	.	8.2	.	.	.	L	B	6	HI	5	LH
157	A	1A	22	LO	.	.	.	.	11.9	349.7	40.0	104	T	A	1	LO	7	HL
158	B	1B	22	LO	.	.	.	.	10.7	472.1	46.0	111	T	B	1	LO	7	HL
159	A	2A	22	HI	.	.	.	.	11.6	198.4	42.5	136	T	A	2	HI	7	LH
160	B	2B	22	HI	.	.	.	.	8.8	205.6	42.0	125	T	B	2	HI	7	LH
161	A	3A	22	LO	.	.	.	.	9.6	208.5	45.5	111	M	A	3	LO	7	HL
162	B	3B	22	LO	.	.	.	.	9.3	271.9	42.5	143	M	B	3	LO	7	HL

OBS	GROUP	STUDENT	DAY	DI	FOLATE	VITC	VITA	FOODFREQ	SF	RBCF	HCT	WT	IML	OCA	STU	DT	DX	ORDER
163	A	4A	22	HI	.	.	.	.	17.5	273.8	43.0	150	M	A	4	HI	7	LH
164	B	4B	22	HI	.	.	.	.	6.9	181.8	43.5	174	M	B	4	HI	7	LH
165	A	5A	22	LO	.	.	.	.	6.9	231.6	42.5	122	L	A	5	LO	7	HL
166	B	5B	22	LO	.	.	.	.	4.8	56.2	41.0	151	L	B	5	LO	7	HL
167	A	6A	22	HI	.	.	.	.	7.2	224.7	41.0	115	L	A	6	HI	7	LH
168	B	6B	22	HI	.	.	.	.	8.5	200.7	46.0	111	L	B	6	HI	7	LH

APPENDIX E

PEARSON CORRELATIONS

VARIABLE	N	MEAN	STD DEV	SUM	MINIMUM	MAXIMUM
FOLATE	12	154.86904762	62.23760379	1858.42857143	75.42857143	285.14285714
VITC	12	97.40476190	44.26136043	1168.85714286	37.00000000	153.28571429
VITA	12	961.28571429	313.25794762	11535.42857143	532.42857143	1375.42857143
FOODFREQ	12	30.21428571	14.73919843	362.57142857	8.21428571	60.71428571
SF	12	10.76944444	6.76572554	129.23333333	2.53333333	22.33333333
HCT	12	42.25000000	3.10058645	507.00000000	39.00000000	50.00000000
RBCF	12	250.30833333	105.43698211	3003.70000000	29.10000000	394.90000000

PEARSON CORRELATION COEFFICIENTS / PROB > |R| UNDER HO:RHO=0 / N = 12

	FOLATE	VITC	VITA	FOODFREQ	SF	HCT	RBCF
FOLATE	1.00000	0.77419	0.47522	0.90594	0.36388	0.01856	0.39629
	0.0000	0.0031	0.1184	0.0001	0.2449	0.9544	0.2022
VITC	0.77419	1.00000	0.79601	0.49685	0.60247	0.18690	0.64343
	0.0031	0.0000	0.0020	0.1003	0.0382	0.5608	0.0240
VITA	0.47522	0.79601	1.00000	0.29482	0.45221	0.27881	0.70159
	0.1184	0.0020	0.0000	0.3522	0.1400	0.3802	0.0110
FOODFREQ	0.90594	0.49685	0.29482	1.00000	0.10102	0.07360	0.26568
	0.0001	0.1003	0.3522	0.0000	0.7547	0.8202	0.4039
SF	0.36388	0.60247	0.45221	0.10102	1.00000	-0.05710	0.72785
	0.2449	0.0382	0.1400	0.7547	0.0000	0.8601	0.0073
HCT	0.01856	0.18690	0.27881	0.07360	-0.05710	1.00000	0.47158
	0.9544	0.5608	0.3802	0.8202	0.8601	0.0000	0.1217
RBCF	0.39629	0.64343	0.70159	0.26568	0.72785	0.47158	1.00000
	0.2022	0.0240	0.0110	0.4039	0.0073	0.1217	0.0000

VITA

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Candidate for the Degree of

Doctor of Philosophy

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