DIETARY FOLACIN AND SERUM FOLACIN IN ADOLESCENT GIRLS

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CHAPTER I

INTRODUCTION

Deficiency states caused by lack of folacin have been investigated in microorganisms, several species of animals, and humans. The dietary essentiality of folacin has been well established (1-3). It is a growth factor for microorganisms and a growth factor and hematological factor for animals and humans.

Many studies of folacin nutritional status of human subjects have suggested that folacin deficiency is more widespread than previously suspected. It is probably the most common hypovitaminosis of man. More cases of folacin deficiency have been reported in pregnant women than in any other population group (4).

Folacin is a nutrient which is required for cell proliferation as well as cell metabolism. It acts as a coenzyme in the metabolism of amino acids and in the synthesis of purines and pyrimidines, which are components of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Therefore, when the number of cells of the body or the rate of cell turnover increases, folacin requirements also increase (3, 5-7). In folacin deficiency, nucleotide synthesis is impaired, and this leads to impairment of DNA synthesis and thus to megaloblastic development of cells. These effects are most noticeable in rapidly growing tissues and cells with high turnover rate, such as the mucosal cells of bucca, gastrointestinal tract, uterus and vagina, and the blood cells. The impairment

of proliferation of intestinal mucosal cells due to folacin deficiency may result in malabsorption of nutrients, including folacin. This further precludes cell regeneration of the intestinal mucosa, establishing a vicious cycle (8, 9).

Folacin is necessary for normal hematopoiesis or formation and maturation of blood cells in bone marrow. It serves as a single carbon carrier in heme synthesis. A late stage of deficiency of folacin in human subject results in the clinical signs of megaloblastic bone marrow and anemia. It is characterized by decreased number of erythrocytes (red blood cells [RBC]), leukocytes (white blood cells [WBC]), and platelets. Clinical and hematologic response is rapid following the oral or parenteral administration of folacin. There is an increase in RBC, WBC, and platelet counts (10). This is followed by a return of the blood morphology to normal (11). Folacin deficiency anemia is manifested in very low serum folacin and RBC folacin concentrations, less than 3 ng/ml and 140 ng/ml, respectively, according to the guidelines in the World Health Organization (WHO) report (12) and the Ten-State Nutrition Survey (13).

Significance of the Problem

Adolescence is a period of time marked by a rapid change in biological, psychological, and social aspects. Biologically, it is a phase of completion of growth and of sexual maturation. Nutrition plays an important role in these processes. An adolescent requires rapid adjustments in nutritional intake to meet the body's need. Failure to meet the additional requirement may result in weight loss, slowed growth and delay of sexual maturation.

The food habits of adolescents seem to be different from those of people at other stages of life. Food consumption and nutritional effects can be greatly influenced by psychological and social factors. The eating patterns of adolescents appear to be unorthodox or random. Snacking for most adolescents is a part of daily behavior (14). For girls, body image is one of the important factors affecting eating, food habits, and nutrition. About half of the adolescent girls in western societies attempt weight reduction by dieting, resulting in the risk of suffering from imbalanced diets (15).

Earlier studies showed that adolescents between the ages of ten and sixteen years had the highest incidence of poor food habits and unsatisfactory nutritional status of all age groups surveyed (13, 16-18). The report of the Oklahoma Nutrition Education Needs Assessment revealved that the percentages of students in the State of Oklahoma who had an "adequate" diet in grades 7-9 and grades 10-12 were only 15.2% and 10.2%, respectively. The "adequate" diet was judged by the minimal standard of including each of the basic 4 food groups at least once in a day (19). These studies have stimulated interest in the folacin nutritional status of adolescents.

As mentioned previously, folacin plays a vital role in DNA synthesis. It is particularly important during periods of increased cell replication and growth such as during pregnancy, infancy, and adolescence. Folacin deficiency may not be as harmful to the adolescent as it is to the fetus (20), since except for the reproductive system, most body tissues have already developed. However, folacin deficiency may result in weight loss, growth retardation, and megaloblastic anemia in adolescents.

Several studies (13, 16, 21-24) have shown that anemia in adolescents is very common, usually resulting from a deficiency of dietary iron. In the United States (U.S.), a high incidence of anemia occurs particularly among blacks (13, 21, 23) and the poor (13). All of the studies cited except the Health and Nutrition Examination Survey (HANES) (16) indicated that the incidence was higher among adolescent girls than among adolescent boys. However, there is very little information available on the occurrence of deficiencies of other nutrients, such as folacin, involved in erythropoiesis. Anemia may be caused by a lack of more than one nutrient at the same time.

Only three studies concerning the folacin nutritional status of adolescent girls have been conducted in the U.S. (25-27). These studies indicate that folacin intakes are often low when compared with the Recommended Dietary Allowances (RDA) (28). One study in 1975 concluded that low folacin intakes in healthy adolescents could, in general, be attributed to incomplete tables of folacin content of food, which gave lower values of folacin, and suggested a need for revision of existing standards (25). In 1977, Perloff and Butrum (29) compiled provisional data on the folacin content in different foods. The newer method they employed to assay the folacin content in selected foods involved protection folacin during the assay and resulted, in most cases, in significantly higher values than those in the Agriculture Handbook No. 29. However, none of the studies reviewed covering dietary folacin intake in adolescents had calculated folacin intakes based on these new data. It is necessary to do more research based on the best available data to confirm whether dietary intake of folacin in adolescent is adequate. Plasma or serum folacin determination may provide a sensitive and early assess-

ment of folacin deficiency (31-36), so serum folacin assay is employed in this study along with dietary evaluation. It is important to find out whether the relationship between dietary folacin and serum folacin of adolescent girls can be affirmed.

Purposes and Objectives

The purposes of this study are to determine the folacin intake from dietary records and the folacin status from serum folacin concentration (SFC) in adolescent girls. The objectives of this study are as follows:

1. To relate dietary folacin to SFC.

2. To relate the intakes of selected nutrients and energy to dietary folacin and to SFC.

3. To relate hemoglobin (HB) concentration to dietary folacin and to SFC.

4. To analyze the influence of race, age, place of residence, height, weight, per capita income, menstrual status, use of nutritional supplements, dieting practices, alcohol consumption, and use of tobacco on dietary folacin and on SFC.

Hypotheses

The following hypotheses were formulated for this study:

Hypothesis 1. There will be no significant relationship between dietary folacin and SFC.

Hypothesis 2. There will be no significant relationship between intakes of selected nutrients or energy and dietary folacin or SFC.

Hypothesis 3. There will be no significant relationship between HB concentration and dietary folacin or SFC.

Hypothesis 4. There will be no significant relationship of race, age, place of residence, height, weight, per capita income, menstrual status, use of nutritional supplements, dieting practices, alcohol consumption, and use of tobacco to dietary folacin and SFC.

Assumptions

The following assumptions are recognized in this study:

1. All respondents give honest responses in 24-hour food intake recalls and questionnaires.

2. Folacin contents of all food items eaten by respondents are included in Nutrition Analysis System (NAS) and are accurate.

3. All forms of folacin in the diet consumed are utilized equally well by respondents.

4. Lactobacillus casei (L. casei) assay of folacin in the serum responds equally to all forms of folacin in the serum.

5. Folacin in the serum is stable and uncontaminated until and throughout the assay.

6. Dietary patterns reported by respondents are typical.

Limitations

The limitations of this study include these:

1. The sample of this study were volunteers from junior high and senior high schools of northcentral Oklahoma.

2. The sample consisted of adolescent girls from 11.5 to 16.5 years of age.

3. Approximately half of the dietary data for this study was collected on Saturdays in the spring of 1981, following an overnight, 10-

hour fast.

4. L. casei was used as test microorganism to assay folacin in the serum of participants in this study.

CHAPTER II

REVIEW OF LITERATURE

There has been a rapid expansion of knowledge and information about folacin over the past 45 years since Wills and Evans (37) first postulated in 1938 that a hematopoietic substance other than antipernicious factor (vitamin B_{12}) for the human existed in crude liver extracts and yeast. In this section, folacin literature is reviewed pertaining to nomenclature, history of discovery, stability, absorption and metabolism, interrelationship with other nutrients and alcohol, deficiency in the human, methods of assessing nutritional status, and intake and requirement in adolescents.

Nomenclature of Folacin

Folacin, which belongs to the vitamin B complex, is used as a generic descriptor for compounds having biological activity and chemical structure similar to those of folic acid (38). It is a term adopted in 1949 by the American Institute of Nutrition as a synonym for folic acid (39). Folate, which is a trivial name given by a scientific group in the WHO, is also a generic term for those compounds (12). The chemical name of folic acid is pteroylglutamic acid (PGA); its chemical structure consists of three components: a pteridine moiety, para-amino-benzoic acid (PABA), and glutamic acid. Chemically related compounds of folic acid exist in the oxidized or reduced state, they carry different single substitutes on the

pteridine moiety, or they have different numbers of glutamyl residues linked by γ-peptide bonds to glutamic acid (38). Although the terms "folic acid" and "folate" have been widely employed and sometimes have been applied to the groups of pteroylglutamates, at other times they have been used to designate a specific compound, PGA, in the literature. Tentative rules of nomenclature for vitamins recommended by the International Union of Nutrition Science Committee on Nomenclature of the American Institute of Nutrition identify the members of the folacin vitamin family (38). The trivial names and abbreviations for folacin members are summarized by Rodriguez (4).

History of Discovery of Folacin

Folacin has been the subject of much investigation involving several animal species, microorganisms, and humans since the 1930's. Research has been very complicated due to the presence of folacin in nature in many chemical forms and in low concentrations, and its susceptibility to destruction or change in chemical forms by heat, light, oxygen, low pH, and folacin conjugase (4, 40).

The diversity of earlier investigations of folacin gave rise to numerous designations for folic acid (PGA) before it was isolated or identified (41). "Folic acid," from the Latin term for leaf (folium), was the name given by Mitchell, Snell, and Williams (42) in 1941 when they extracted from spinach the substance which promoted the growth of L. casei. In 1945, PGA was synthesized, and it was confirmed to be the same chemical structure as folic acid or L. casei factor by Angier and his coworkers (43). Synthetic PGA has been used in much research concerning folacin absorption and metabolism since then. The history of the

discovery, isolation, and identification of various form of folacin has been thoroughly reviewed by Jukes and Stokstad (44). It is also described in detail by Wagner and Folkers (45, p. 114), who state that the history of folic acid is "the most complicated chapter in the story of the vitamin B complex."

Factors Affecting Stability of Folacin

Although all members of the folacin family may be shown to possess biological activity, they vary widely in stability. In general, physical factors such as heat and light destroy folacin activity, and chemical factors such as low pH and oxidation decrease the stability of folacin.

Folacin is destroyed by temperature greater than 125°C (36). Food folacin activity may be destroyed to the extent of 50-95% by cooking and/or heat processing (46). Folacin in animal foods shows smaller losses than that in green vegetables (47). Free folacin is more easily destroyed than bound folacin during food preparation (6, 29). Milk folacin activity was destroyed to the extent of 17-74% by drying, terminal sterilization, or boiling; and reheating led to even greater losses (48).

Folic acid is sensitive to light. Photodecomposition of a folic acid solution by sunlight to yield PABA and pterin was first reported by Stokstad, Fordham, and Grunigen (49).

All folacins in the completely reduced form, except formiminotetrahydrofolic acid (THF), are rapidly oxidized by air or other oxidizing reagents (50-53). Ascorbic acid can protect reduced folacins from oxidative and thermal destruction, and its protective action of oxidation may be due to the removal of dissolved oxygen (31-36, 50, 53).

Comparing the stability of different forms of folacin in an acid pH, THF shows the greatest stability, but N^5-formyl THF, N^{10}-formyl THF, and formimino THF are readily converted to 5,10-methenyl THF (also called anhydroleucovorin, anhydrocitrovorum factor, or the acid degradation products of folinic acid) (53, 54). Folic acid and methyl THF are also unstable in an acid pH.

Polyglutamyl forms of folacin, also called folacin conjugates, are hydrolyzed under alkaline and anaerobic condition to yield folic acid and glutamic acid (55). N^5-formyl THF shows considerable stability to alkaline hydrolysis, in contrast to N^{10}-formyl THF, which readily loses its formyl group in alkali (52). Formimino THF is readily hydrolyzed with loss of ammonia (53). O'Broin and his coworkers (50) suggested that polyglutamates which contain extra glutamyl residues are unlikely to affect the nutritional instability associated with pteroylmonoglutamates.

Absorption and Metabolism of Folacin

Folacin is present in a wide variety of foods. Food folacin exists as a mixture of free and conjugated forms, with the latter predominating (29). Conjugated folacins are not reflected in the microbiological assay without prior treatment with an enzyme known as conjugase (also called pteroylpoly-γ-glutamyl hydroxylase or pteroylpolyglutamyl carboxypeptidase). "Free folacin" is a term representing those forms of folacin that can promote the growth of microorganisms when assayed microbiologically without prior treatment with conjugase. It includes mono-, di-, and tri- glutamyl forms of folacin (25, 56). However, free folacin is often used as a synonym for monoglutamyl forms of folacin in the literature.

The pathways for the absorption of monoglutamyl and polyglutamyl forms of folacin from foods appear to be separate (41, 57-59). Different monoglutamates are absorbed with different effectiveness in the human, measured by increases in SFC after feeding (60). The hydrolysis of polyglutamates to monoglutamates during the process of intestinal absorption is catalyzed by conjugase in the intestine. The possible sites of hydrolysis include intraluminal contents, the brush border surface of the intestinal mucosa, or the interior of the mucosal cell (41, 57-59). Butterworth and Krumdieck (58) concluded from the review of the research on intestinal absorption that polyglutamyl forms of folacin are absorbed intact by intestinal epithelial cells and cleaved to monoglutamates at an intracellular site before passage across the intestinal cell membrane into the circulating blood.

Several factors affecting folacin absorption are the chemical structure of folacin, pH, a naturally occurring inhibitor of folacin conjugase, glucose, fiber content of the diet, folacin antagonistic substances like alcohol and some drugs, and folacin deficiency itself. Uptake of PGA by the perfused rat jejunum, optimal at pH 6, could be significantly decreased by either acidification or alkalinization of the perfusate (61). Yeast contains a conjugase inhibitor which makes the folacin in yeast unavailable (62, 63). Cellulose and other fibers bind to monoglutamates and make them unavailable for absorption (64). However, this is not confirmed by other studies (65, 66). Glucose increases folic acid absorption in the perfused human jejunum (67). Impaired jejunal uptake of folacin has been demonstrated clinically in alcoholics (68).

The proximal small intestine, the jejunum, is the major site of folacin absorption. In one study, the absorption of perfused crystalline PGA in humans occurred throughout the small intestine with maximal absorp-

tion in the jejunum (69). The absorption of polyglutamyl forms of folacin needs an intact jejunum. These forms might not be absorbed in the ileum (41, 57, 58).

There are two separate and distinct absorption mechanisms for folacin. The absorption of small (physiological) quantities is an active, energy-requiring process, whereas large quantities are absorbed by diffusion (70, 71). Pteroylglutamates are reduced and methylated to form methyl THF in the jejunal mucosa during the absorption process. This is demonstrated by the rise of blood folacin supporting the growth of L. casei but not supporting that of Streptococcus faecalis (S. faecalis) and Pediococcus cerevisiae (P. cerevisiae) after feeding different forms of folacin (60).

In humans, the form of folacin circulating in the blood is the monoglutamate with methyl THF predominating (60, 72). Serum folacin seems to exist in two states: free and bound to albumin, α_2-macroglobulin, or probably transferrin (73). Most oxidized folacin in serum is protein-bound specifically, while reduced folacin, such as methyl THF, is free or weakly nonspecifically bound. The latter likely represents the route for the transfer of biologically active folacin from one tissue to another (6). It was confirmed that a significant amount of methyl THF binding could be accounted for by albumin (74).

Folacin in circulating blood is removed to the intracellular space rapidly as determined by both plasma clearance and urinary folacin which indicates that not much folacin is excreted after feeding folacin (60). Neal and Williams (75) found that 24 hours after intravenous injection of tritiated folacin into rats, half the unexcreted radioactivity could be accounted for in the liver, kidney, spleen, testes, and duodenum.

Folacin is found in all organs, tissues, and body fluid. Liver is the major storage site of folacin. It holds reserves of from 5 mg to 10 mg of folacin in humans (76). More than 85% of liver folacin is present as pteroyl-polyglutamates (77). The concentrations of folacin in brain and cerebrospinal fluid are 2-3 times higher than those found in serum even in states of folacin deficiency. This indicates that folacin has a special role in the central nervous system (78).

Different reduced forms of polyglutamate folacin, rather than monoglutamates, are preferentially retained by tissues (79). They act as coenzymes in all biological reactions involving the transfer of single carbon units except carbon dioxide. Those biological reactions are summarized on a flow chart of folacin metabolism (80). In summary, folacin coenzymes are involved in (i) metabolism of purines and pyrimidines which are components of DNA and RNA; (ii) metabolism of amino acids such as serine and glycine, phenylalanine and tyrosine, and histidine; (iii) biosynthesis of methyl groups such as those in methionine and choline. They methylate small amounts of transfer RNA and initiate protein synthesis in procaryotes (81). They are also involved in the metabolism of long-chain fatty acids in the brain (82) and lipids such as phospholipid and triacylglycerol in the liver (83).

Interrelationship of Folacin with Other Nutrients and Alcohol

Vitamin B_{12}

Both vitamin B_{12} and folacin are required for synthesis of thymidylate, and therefore, of DNA. They also have closely connected metabolic functions in hemopoiesis. Deficiency of either or both produces

megaloblastic anemia (84). Vitamin B_{12} affects the transport, metabolism, and storage of folacin in cells. Vitamin B_{12} deficiency causes a profound disturbance of those. It may be described by the most plausible theory as a "methyl folate trap": vitamin B_{12} is needed as the coenzyme of methyl THF homocysteine methyltransferase which catalyzes the conversion of methyl THF to THF. Only THF is the preferred form taken into cells. In deficiency of vitamin B_{12}, there is an accumulation of methyl THF in the blood, which is metabolically useless and causes the depletion of cells of other forms of folacin that are active in nucleic acid synthesis (85, 86). Those may be shown by increased serum folacin and decreased RBC folacin in vitamin B_{12}-deficient patients (86, 87). The liver folacin storage is also decreased in vitamin B_{12}-deficient animals (88, 89).

Vitamin C

Ascorbic acid is a reducing agent which protects folacin against oxidative and thermal destruction (31-36, 50, 90). Deficiency of vitamin C affects the metabolism of folacin and may affect folacin requirement. Megaloblastic anemia may occur in scurvy (91-94). In some cases, vitamin C alone has corrected the anemia (91, 92), while others require folacin (93, 94). It was found that the major folacin compound in the urine of the scorbutic patient with megaloblastic anemia was N^{10}-formyl folic acid. After treatment with vitamin C, 5-methyl THF became predominant. It was postulated that the anemia of scurvy is due partially to the depletion of folacin pool and that an important role for vitamin C in human metabolism may well be to reduce the rate of oxidation of N^{10}-formyl THF and thereby keep the folacin metabolic pool replete (94).

Iron

The incidence of combined deficiency of iron and folacin is high (95-99), especially in pregnant women (97-99). The interrelationship between iron and folacin is complex. Both affect hemoglobin concentration (95, 96). It has been suggested that iron deficiency increases RBC folacin (100) and decreases plasma folacin (101) or does not change the amount of plasma folacin (100). Iron deficiency appeared to increase the folacin requirement (101). In folacin deficiency, iron absorption was increased (102) and iron stores were increased (103). Excess iron injected into women with severe folacin deficiency was toxic (104).

Zinc

The experimental zinc deficiency in the human decreased the absorption of polyglutamyl forms of folacin, suggesting a specific role of dietary zinc in the hydrolysis of pteroyl-polyglutamates (105). Bovine hepatic folacin conjugase is a zinc metalloprotein (106). This implies that intestinal conjugase may be also a zinc-containing enzyme and that zinc may be required for the absorption of dietary folacin.

Riboflavin

Riboflavin affected the utilization of folacin for the biosynthesis of coenzyme derivatives, particularly in the reduction steps responsible for the conversion of folic acid to THF (107). Riboflavin had a positive effect on hemoglobin concentration as well as hematocrit value and RBC count when it was administered with folacin to the rat. This effect might have been partially due to the effect of riboflavin on folacin metabolism (108).

Methionine

There was decreased uptake of ^{3}H-folic acid by liver (89) and decreased amount of liver folacin (109) in methionine-deficient rats. Dietary methionine rearranged the folic acid distribution pattern in the liver of the rat (79, 86). Buehring et al. (110) found that methionine changed the form of folacin in the liver of rats. It decreased the quantity of 5-methyl THF and increased the proportion of other monoglutamates and polyglutamyl forms of folacin.

Glucose

The only study concerning glucose and folacin indicated that glucose enhanced the absorption of folacin fed at the same time (66).

Alcohol

Various degrees of folacin deficiency are commonly encountered in alcoholic populations (111). Contributory causes of alcoholic folacin deficiency include low dietary folacin intake, inadequate hepatic storage (112), and intestinal malabsorption ingestion (111). The type of alcoholic beverage and the amount of alcohol ingested also affect the degree of folacin deficiency (111).

A direct relationship between alcohol and folacin-deficient anemia was first established by Sullivan and Herbert (113). When they administered alcohol to three patients with megaloblastic anemia due to folacin deficiency, the response to orally or parenterally administered folacin was interrupted by simultaneous administration of alcohol.

Alcohol ingestion generally reduces the absorption and tissue uptake of folacin, and induces a fall in circulating folacin. The mean

folacin concentration in the serum in alcoholics tended to be lower than in the control population (111). Even short-term ingestion of a relatively small amount of alcohol depressed the serum folacin in normal subjects within a few hours, and the degree of this depression seemed to correlate with the amount of alcohol consumed (114). This was interpreted to mean that alcohol blocked the mobilization and transport of methyl THF from tissue stores to blood (111).

Another study of the effect of prolonged alcohol feeding on hepatic and intestinal folacin metabolism using monkeys as a model indicated that the hepatic folacin concentration in the alcohol-fed group was significantly less than in the control group (115). Continued alcohol feeding might result in progressive hepatic injury which might be associated with more severe impairment of folacin metabolism. Intestinal hydrolysis of polyglutamates was not impaired by chronic alcohol feeding. The activity of jejunal folacin conjugase was not diminished by alcohol, but the absorption of pteroylmonoglutamate was decreased (115).

Folacin Deficiency in the Human

As with all avitaminoses, deficiency of folacin may occur from inadequate intake, defective absorption, deranged utilization, increased requirement, increased destruction or increased excretion (116). Earlier studies of folacin nutritional status in adolescent girls showed there was a high incidence of low intakes of folacin, based on calculating folacin content of the diet eaten from old food composition tables and comparing with the RDA (25-27). It is possible that folacin deficiency occurs most commonly from inadequate dietary intakes.

Folacin deficiency is characterized by megaloblastosis of the bone marrow, macrocytic (megaloblastic) anemia, diarrhea, and glossitis.

Weight loss is frequent. All of the signs associated with anemia and tissue anoxia may develop, including weakness, syncopal attack, and pallor of skin (5). Sleeplessness, forgetfulness, and irritability also occur (117). Folacin deficiency may also have an effect on the nervous system, peripheral neuropathy, organic dementia, and posterior lateral column lesions have been reported to be caused by nutritional folacin deficiency (118).

Experimental deficiency of folacin in the human was induced by Herbert (117) using a diet that furnished 5 μg of folacin daily for 4.5 months. The biochemical and hematological sequence of events in developing folacin deficiency in this study were as follows: After 3 weeks of folacin deprivation, folacin activity dropped to less than 3 ng/mg; after 7 weeks, hypersegmentation of the polymorphonuclear leukocytes was observed. After 14 weeks, there was an increase in excretion of formiminoglutamic acid (FIGLU) in the urine. After 17 weeks, the folacin content of RBC decreased, and shortly after this macroovalocytosis appeared. In the nineteenth week, the bone marrow became megaloblastic and mild anemia developed rather suddenly. All of the abnormal findings responded rapidly to the administration of folic acid. This study indicated the SFC fell rapidly to below-normal concentration well before the appearance of hematological change. The first hematologic abnormality was an increased average number of lobes of the nuclei of the polymorphonuclear leukocytes, with five or more, rather than the normal average of 3.2. Morphological change in the peripheral blood occurred before the appearance of an overt megaloblastic bone marrow.

Deficiency of folacin occurs commonly in some clinical conditions. These include macrocytic anemia, neurological disease, alcoholism,

liver disease, gastrointestinal disease such as tropical sprue, celiac disease (also known as gluten-sensitive enteropathy), villous atrophy, systemic bacterial infections, dermatitis herpetiformis, and regional enteritis, surgery of intestine or resection of jejunum, neoplastic disease, food faddism, drug abuse, and pregnancy (6, 84).

Methods Assessing Folacin Nutritional Status

Folacin nutritional status can be evaluated with reliability by biochemical techniques. A number of procedures have been proposed such as assay of folacin concentration in the serum or plasma, whole blood, RBC, leukocytes, or liver biopsy specimens; formiminoglutamic acid (FIGLU) test (35); plasma folacin clearance test and absorption test (59, 119); and deoxyuridine suppression test (120).

Of those tests available for assessment of folacin status, the most commonly performed and most practical for application to population studies is the measurement of serum or plasma folacin concentration by microbiological assay with L. casei. It provides the best and earliest method of assessing folacin deficiency but provides little information concerning tissue stores. Low serum or plasma folacin concentration may not necessarily be associated with megaloblastic anemia nor with any other biochemical changes (117). It may be due to a negative folacin balance caused by recent low dietary intake of folacin (117) or increased folacin requirement in case of infection (6, 84). However, continued low serum or plasma folacin with continued depletion would eventually be accompanied by signs of megaloblastic anemia and megaloblastic bone marrow changes (117). The folacin concentration of plasma is almost the same as that of serum (33, 90).

Folacin stability in the serum or plasma is a major consideration during storage and in the course of assay. Without any added ascorbic acid, serum or plasma loses part of its folacin activity during storage at -20°C (121, 122). Usually, a small amount of ascorbic acid is added to protect folacin activity in the serum or plasma from oxidative destruction. The protective effect of ascorbic acid was the same with concentrations between 5 and 15 mg per ml of serum. Other studies showed that 1 or 1.5 mg of ascorbic acid per ml of serum was enough (33, 121). The added ascorbic acid had no effect on the folacin activity of fresh serum (121). According to O'Broin et al. (90), serum or plasma folacin stored in a 5 mg/ml solution of ascorbic acid at -20°C was stable for years. Bird et al. (123) have demonstrated that freezing and thawing cause deconjugation and oxidation of folacin in the blood and liver, and the methyl form is converted to the more stable N^5-formyl form.

RBC folacin concentration has been regarded as a more accurate and less variable quantitative index of the severity of folacin deficiency than serum folacin (35, 124). The RBC folacin reflects the body folacin status at the time RBC were formed (6, 35). Nevertheless, low RBC folacin does not distinguish between megaloblastic anemia due to a lack of vitamin B_{12} and that due to a folacin deficiency. Both low serum folacin and low RBC folacin are strong evidence that a folacin deficiency exists (117, 124). Hoffbrand et al. (124) described a microbiological assay and determined the folacin content of RBC in 40 healthy subjects and 120 patients with subnormal SFC due to several clinical conditions. They found a good correlation between RBC folacin and severity of folacin deficiency as assessed by polymorphonuclear lobe counts or the bone marrow morphology in non-

anemic patients. They concluded that in the absence of vitamin B_{12} deficiency, the RBC folacin concentration was a precise guide to the severity of folacin deficiency.

Another frequently used biochemical parameter of folacin deficiency is the FIGLU test. FIGLU, an intermediate in the conversion of histidine to glutamic acid, loses its formimino group to THF and results in the formation of glutamic acid and N^5-formimino THF. In folacin deficiency, this step of histidine catabolism is blocked and the excretion of FIGLU is increased in the urine (35, 80). The amount of FIGLU excreted appears to be a satisfactory index of tissue folacin stores (124). However, FIGLU test is not entirely specific for detecting folacin deficiency, since many factors such as iron deficiency (125, 126), hemolytic anemia, neoplastic disease (126), liver disease, protein malnutrition, congenital formiminotransferase deficiency (35), vitamin B_{12} deficiency (35, 124), and thyrotoxicosis (127) cause an abnormal excretion of FIGLU. It would be better used as a confirmary test than as diagnostic test.

Microbiological assay is commonly used to measure folacin concentration in the serum or plasma, RBC, urine, and other biological material, although radioisotopic assays have been developed to measure serum folacin since 1971 (128). The accuracy of the latter has been questioned. It costs more than microbiological assay.

The microorganisms most commonly employed for assay have been L. casei, S. faecalis, and P. cerevisiae. They respond to different degrees to various forms of folacin. L. casei responds to mono-, di-, and triglutamates of PGA and reduced pteroylglutamates; S. faecalis, to these folacins with the exception of methyl forms of folacin; and P. cerevisiae, to completely reduced formyl forms of folacin (4, 34, 129).

There are some advantages and disadvantages of microbiological assay. The advantages include these: it can accurately detect minute amounts of nutrients like folacin not possible by traditional chemical methods; it is relatively simple to set up; and the cost is economical. The disadvantages are: it is a time-consuming process; it is interfered with by antibiotics such as tetracycline which suppress L. casei growth (3, 31) and by other drugs; it does not provide precise information on the kind or the amount of the individual folacin derivatives in the sample; and the growth-supporting activity for bacteria does not necessarily indicate that for the human (1, 3, 4).

Folacin Intake and Requirement in Adolescents

The estimates of the total folacin content of foods and meals vary over a wide range from one study to another (6). American diets assayed by L. casei as test organism contained between 37 and 297 μg, with an average of 149 μg. They contained 379 to 1097 μg of total folacin with an average of 689 μg when treated with conjugase according to Butterfield et al.(56). Another study indicated that free and total folacin assayed in diets collected at home were in a range of from 63 to 586 and 69 to 601 μg, respectively. Mean free folacin was 206 μg per day (median 129 μg); and mean total folacin was 242 μg per day (median 158 μg) (130).

Three earlier studies showed that folacin intakes of adolescent girls were far below the RDA (25-27). One indicated that more than 85% of girls took in less than 1/3 the RDA (25-27). The other two reported that more than 90% of girls consumed less than 50% of the RDA (25, 27), and about half of the girls ingested less than 10% of the RDA for folacin (27).

The quantitative needs for folacin in the human have not been clearly established. The assay of folacin activity in foods is still uncertain, although the latest analytical data on the folacin content of food has made feasible more accurate estimates of the folacin content of diets than were possible earlier (29). The folacin content of the diet, as eaten, is greatly influenced by the method of cooking. Cooking and/or processing may result in losses of food folacin activity as high as 50-95%, and even 100% when high temperatures and large volumes of water are used (46). Even if the amount of folacin present in food can be determined, there is still the problem of folacin availability for absorption. The availability of folacin varies from one food to another, which may be due to the presence of conjugase, conjugase inhibitor, or folacin binder, as mentioned previously. Those factors affect folacin available to the human from food.

There is no information available concerning the requirement of folacin for adolescents specifically, according to a literature review. However, one study concerning the minimal daily folacin requirement for adult females conducted by Herbert (131) may provide information as reference. For three normal women receiving a diet containing approximately 5 μg folacin daily, serum folacin fell clearly below normal (equal to or above 6 ng/ml) by the end of 40 days in one subject receiving 25 μg of synthetic PGA. It did not fall significantly in another subject receiving 50 μg, and it remained clearly normal in the other subject receiving 100 μg daily. Herbert suggested that the minimum requirement of folacin for the adult female may be between 50 and 100 μg per day.

In setting the RDA, the National Research Council assumed 25% absorption in order to allow a wide margin of safety for the difference of availability from various food sources. The RDA for adults and adolescents was set at 400 µg of "total folacin" activity in the diet (28). An FAO/WHO Expert Group recommended that the dietary intake of "free folacin" for adolescents should be 200 µg per day (12).

CHAPTER III

METHODS AND PROCEDURES

The research was designed to achieve the objectives of this study. After subjects were selected, data necessary to the study were obtained by interviews with subjects and their mothers, and by chemical and microbiological assay, and data were analzed by appropriate statistical procedures.

Research Design

Descriptive survey involving various kinds of data was the design for this study. Descriptive research looks at differences or relationships between independent variables and dependent variables. Data necessary to this study included dietary information, anthropometric measurements, demographic data, supplemental nutrients, dieting practice, alcohol consumption, use of tobacco, and concentrations of serum folacin and other blood components.

Population and Sampling

Adolescent girls who were residents of northcentral Oklahoma constituted the population of this study. The sample was obtained on a voluntary basis from selected area in northcentral Oklahoma, mostly by contacts through public schools. Most of the subjects attended schools in Perry, Tulsa, Stillwater, or Perkins. Subjects were selected on the basis of race (white or black) and age (11.5-16.5 years). A

total of 150 girls participated in this study.

Instruments

Interviews and questionnaires which were developed for the S-150 Southern Regional Project, "Nutritional Health of Adolescent Females," provided much of the data necessary to this study. Questionnaires for 24-hour food intake recall gave the dietary information about the subject. A medical history and anthropometric measurements of the subject, and socio-demographic background information from the subject's mother provided data for independent variables in this study. Those questionnaires are shown in Appendix A.

Data Collection

During the spring of 1981, several mornings (February through May, usually on Saturdays) were spent in major data collection sessions. The places of data collection were arranged in Perry High School, Oklahoma State University, and a clinical center in Tulsa. When subjects came in and registered, all questionnaires were distributed to them. Then they were guided to various data collection stations as stations were available.

Dietary Intake Information

Dietary information was obtained through interviews by trained nutritionists and recorded on the appropriate form for 24-hour food intake recall (see Appendix A - Form D 1b). On the day of central data collection, subjects were asked for the food items and amounts of foods eaten. Food models and containers for liquid and solid food were used

to assist subjects to report accurately the amounts of food eaten. The subjects were also asked if they had taken a nutritional supplement. A second dietary recall was done at least two weeks before or after the main data collection day. Nutrient intakes were calculated by the Nutritional Analysis System (NAS)[1], a computerized data bank.

Anthropometric Measurements

Balances used to weigh subjects were checked against each other. Before height and weight measurements each day, the physician's scale was reset to zero. The subjects were asked to take off coats and shoes, stand upright, and face to front on the scale. The height and weight were measured carefully and recorded on Form A3 - A (Anthropometric Measurement Sheet) (see Appendix A). Height was read to the nearest quarter-inch, and weight was read to a quarter-pound. Items of clothing were listed, and their weights were estimated from a list of weights of similar items of clothing. Then the body weight was determined by subtracting the weight of clothing from the weight of the subject initially recorded.

Socio-Demographic Data

Information such as age, race, place of residence, and per capita income was obtained from subject's mother by interview and recorded on Form S-1 (see Appendix A). Other information such as menstrual status, alcohol consumption and smoking tobacco, was obtained by interview with the subject and recorded on Form A2 (see Appendix A); dieting

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[1]At Louisiana State University, Baton Rouge.

practices were recorded on Form D1 (see Appendix A).

Blood Collection

Fasting blood was withdrawn from the subject in vacutainers for determination of SFC and other biochemical tests. Blood was chilled immediately and kept in dim light at all times.

Folacin Assay

Sample Preparation

Blood was centrifuged for 20 minutes at 2500 rpm (relative centrifugal force =1242 G [gravity]) at 5°C. About 0.5 ml of the supernatant serum was transferred into a small clean vial containing 0.5 mg dry ascorbic acid. All serum samples were protected from light and were frozen at -20°C until assay.

Maintenance of Assay Organism and Preparation of Inoculum

Folacin assay by microbiological method used standard sterile technique (132). The test organism was L. casei, American Type Culture Collection[2] (ATCC) No. 7469, obtained in freeze-dried form. Accompanying instructions were followed to establish an actively growing culture in liquid medium. Bacto-Lactobacilli Broth AOAC[3] was used. After recovering L. casei, stab cultures were grown in the agar and maintained at 4°C.

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[2]12301 Parklawn Drive, Rockville, Maryland.

[3]From Difco Laboratories, Detroit, Michigan.

These cultures were prepared to be used in case the test organism later became contaminated. L. casei was also maintained as a liquid culture which was transferred from agar culture and incubated 18 hours at 37°C, then refrigerated. At a maximal interval of once a week, one drop of each stored liquid culture was added to 5 ml of fresh broth, incubated 18 hours at 37°C, and then stored at 4°C.

On the afternoon before setting up an assay, one drop of the latest stored culture was added to 5 ml of maintenance broth, and incubated 18 hours at 37°C. The next morning, 0.5 ml of this fresh 18-hour culture was added to 5 ml of maintenance broth and incubated 6 hours at 37°C. The inoculum for assays was then prepared with this culture in two steps. First, the culture was centrifuged and the supernatant solution was decanted aseptically. Second, the cells were resuspended in 5 ml of single-strength basal medium and centrifuged; then the supernatant solution was decanted. The second step was repeated four more times. Finally, the washed pad of organisms was resuspended in 5 ml of single-strength basal medium. One drop of this inoculum would be added to each assay tube.

Preparation of Single-Strength Basal Medium for Assays

The assay medium for L. casei was Bacto Folic Acid Casei Medium[4]. Supplier's directions were followed to prepare the basal medium. Since an equal volume of distilled water was added to rehydrate the medium, the concentration of nutrients in this single-strength basal medium was half of the original as purchased. Four hundred milliliters of the

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[4]From Difco Laboratories, Detroit, Michigan.

single-strength assay medium was generally prepared at one time and dispensed into 80 sterile tubes.

Preparation of Stock Standard Folic Acid Solution

Folic acid (PGA)[5] was dried in a desiccator and stored in a freezer. It was removed two hours before standard preparation and allowed to come to room temperature. Ten milligrams were diluted to 100 ml with 0.5% sodium ascorbate. This Standard I contained 100 µg folic acid per ml solution. Standard II was made by diluting 1 ml of Standard I to 100 ml in 0.5% sodium ascorbate and contained 1 µg per ml. A 100 ng/ml folic acid solution (Standard III) was made by diluting 10 ml of Standard II with 0.5% sodium ascorbate to 100 ml. This was dispensed in 4-ml quantities into test tubes and stored in the dark at -20°C.

Standard Curve

On the day of the assay, one tube of Standard III was removed from the freezer and allowed to thaw in the dark. Concentrations for standard curve included 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 ng/ml. Two milliliters of 0.5% sodium ascorbate was pipetted into each of the test tubes, and 2 ml of Standard III (100 ng/ml folic acid solution) were added to the first of these test tubes, then it was mixed thoroughly. This tube contained 50 ng/ml folic acid. Then 2 ml of this first dilution were added to the next tube of 2 ml of 0.5% sodium ascorbate to give 25 ng/ml folic acid solution. By continuing this double dilution

[5]Sigma No. 7876. Sigma Chemical Company, St. Louis, Missouri.

process, 4 other concentrations of folic acid solution were made.

Setting up the Assay

Each assay was done in duplicate. One-tenth milliliter of serum or folic acid standards was pipetted aseptically into tubes containing 5 ml of fresh folic acid casei medium. All tubes were mixed thoroughly on a Votex mixer. Then one drop of inoculum from a sterile Pasteur pipette was added to each tube and all test tubes were incubated 18 hours at 37°C.

Calculating the Assay Results

After an 18-hour incubation, each tube was shaken thoroughly and left standing for 15 minutes before reading absorbance in a spectrophotometer at 660 nm. At that wavelength, the color of the assay broth had a minimal effect upon the turbidity readings. The instrument was set to zero absorbance with an inoculated blank instead of uninoculated one, since the latter read lower than the former. Then the absorbance of each serum sample and folic acid standard was read and recorded. The average of the duplicates was taken to calculate the results.

Standard curves were plotted on semilogrithmic paper using absorbance x 1000 as the ordinate and the concentration of folic acid as the abscissa plotted on the log scale. The reasons for using a semilogrithmic plot rather than a regular plot are that the process of drawing straight lines is objective; it is easy to make critical evaluation of data; the usable portion of the curve is long; calculation can be done more precisely and quickly (133). The folacin activity of serum was calculated from the standard curve relating the amount of folic acid to

absorbance of the standard. In this study, the standards were usually treated as two straight lines, with low concentrations (below 6.25 ng/ml) calculated from one line, and the concentrations above 6.25 ng/ml from the other.

Data Analysis

Dietary intake from 24-hour food intake recalls was converted from measurements of food or numbers of units to weight of food. Then the quantities of folacin, other nutrients, and energy in the diets of each subject were calculated by NAS. The average intake of each nutrient and energy was taken from the two recalls. Results were expressed as the absolute values. The mean, standard deviation (S.D.), and the range of folacin intakes from all subjects were computed and presented. Further, the folacin intake of each subject was expressed as a percent of the RDA (28). All subjects were classified into three groups according to their RDA folacin percentage: marginal-to-adequate folacin intake, above or equal to 2/3 RDA; low folacin intake, between 1/3 and 2/3 RDA; and very low folacin intake, below 1/3 RDA.

The values of serum folacin activity for L. casei were interpreted according to the guidelines used in a WHO report (12) and the Ten-State Nutrition Survey (13). Less than 3.0 ng/ml is considered indicative of folacin deficiency; 3.0-6.0 ng/ml is low; and equal to or above 6.o ng/ml is normal. All subjects were classified into two groups according to whether or not they took antibiotics in the week preceding the blood-drawing day, since antibiotics might interfere with serum folacin assay and result in a lower apparent serum folacin value. The mean, S.D., and the range of all SFC of those two groups and total subjects were computed

and presented. The data for subjects who took antibiotics were excluded from the further statistical analyses.

The Pearson Product Moment Correlation Coefficients (134) were calculated to determine the relationships among variables, including dietary nutrients, SFC, and HB concentration. Two races were white and black. Three age groups were younger than 13.5 years, between 13.5 and 15.5 years, and older than 15.5 years. Three groups by the place of residence were city, town, or rural area, which were defined as follows: a city of population greater than 100,000, towns of population between 2,500 and 100,000, and rural areas or small towns of less than 2,500. The girls were divided into the groups of as nearly equal size as possible according to their heights and weights. Three height groups were below 156.2 cm, 156.2-161.3 cm, or above 161.3 cm. Three weight classifications were below 46.2 kg, 46.2-54.9 kg, or equal to or above 54.9 kg. Per capita income was divided into the yearly income of under $2,700, of $2,700 to 5,700, and of over $5,700 in order to have at least the minimal numbers of white and black girls in each income group. Pre-menarcheal and post-menarcheal girls were compared, as well as subjects who did or did not use nutritional supplements, consume alcohol, or smoke tobacco. The least squares mean (LSM) folacin intakes, and SFC for groupings based on each of those 11 independent variables were computed. The analysis of variance on the basis of a general linear model (GLM) of the Statistical Analysis System (SAS) (134) was used to determine the effects of each independent variable on the dietary folacin and SFC. T or F tests were used to identify the significant differences in the folacin intake and SFC among the groups due to each independent variable. Further, for each independent variable, the frequencies

and percentages of subjects in each category were computed according to the categories of folacin intakes relative to the RDA, and according to the deficient, low, or normal SFC. Then a chi-square test was used to determine the significance of any association between the independent variable and the folacin intake or SFC. Subjects with the SFC above 30.0 ng/ml or HB concentration[6] below 12.0 g/dl, or mean corpuscular volume (MCV)[6] above 85.0 fl were further examined in relation to the nutrients thought to be involved in hematopoiesis, including folacin, vitamin B_{12}, iron, and riboflavin. The SFC and serum ferritin[6], an iron-containing protein which reflects the iron stored in the body, were employed for accurate examination of the causes of low HB concentration rather than the dietary folacin and iron.

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[6]Data are from the Research Laboratory of the Department of Food, Nutrition and Institution Administration, Oklahoma State University.

CHAPTER IV

RESULTS AND DISCUSSION

In this chapter, the results are presented and discussed. Conclusions are drawn on the basis of the original objectives. One hundred and fifty girls were involved in this study.

Intake of Folacin

Two 24-hour recalls of food intake for each subject were analyzed for many food components, including vitamin B_{12}, vitamin C, iron, zinc, riboflavin, methionine, sucrose, and energy as well as folacin. The average for the two days was taken. The individual nutrient and energy intakes as well as other variables for each subject are shown in Appendix B.

The folacin intakes of 150 girls ranged from 24 µg (7% RDA) to 831 µg (208%RDA). The mean was 215 µg (54% RDA) (Table I). Fifty-six girls had marginal-to-adequate intakes, defined as equal to or above 2/3 RDA; forty-nine had low intakes, between 1/3 and 2/3 RDA; and forty-five had very low folacin intakes, below 1/3 RDA (Table II). Folacin intakes of many girls in this study were low when compared with the RDA, although the average amount of folacin in two 24-hour recalls was based on the current data for the folacin content in the selected foods, which gave higher values for folacin than those used previously from the USDA Agriculture Handbook No. 29 (135). The prevalence of

TABLE I

MEAN FOLACIN INTAKE AND SERUM FOLACIN OF ALL SUBJECTS AND OF SUBJECTS BY USE OF ANTIBIOTICS

Folacin Intake (μg)				Serum Folacin (ng/ml)			
N	Mean	S.D.	Range	N	Mean	S.D.	Range
150	215	143	24-831	144*	11.9	12.3	0.5-68.4
142+	216	144	24-831	136+	12.2	12.5	0.5-68.4
8‡	191	139	30-413	8‡	6.6	6.0	0.6-20.0

*The total number of subjects having the blood available for serum folacin assay.

+The total number of subjects not reporting taking antibiotics.

‡The total number of subjects taking antibiotics within the week preceding the blood-drawing day.

TABLE II

DISTRIBUTIONS OF FOLACIN INTAKE BY RDA CATEGORIES

Folacin Intake	n	Percent
< 1/3 RDA*	56	37.3
1/3-2/3 RDA	49	32.7
≥2/3 RDA	45	32.0

*RDA = 400 μg.

low intake confirmed the earlier studies (25-27) in which the adolescents reported taking in low intakes of folacin. In this study, 57% of the girls consumed less than 50% of the RDA, a lower percentage than the 90% in two studies (25, 27); and 2.7% took in less than 10% of the RDA, which was also lower than 50% of the girls reporting less than 10% of the RDA in one of those studies (27). Only about one-third of the girls in this study had folacin intakes above 2/3 the RDA, which was arbitrarily defined as marginal-to-adequate.

Serum Folacin

Blood samples were available for serum folacin assay for 144 girls in this study. SFC of all girls ranged from less than 1 ng/ml to 64.8 ng/ml. The mean was 11.9 ng/ml (Table I). Eight of the girls had taken an antibiotic within the past week (Table I). Of all the sample, 26 girls had folacin deficiency (SFC below 3.0 ng/ml), 27 girls had low SFC (3.0-6.0 ng/ml), and 91 were normal (SFC equal to or above 6.0 ng/ml) (Table III). The mean SFC for the girls taking antibiotics was 6.6 ng/ml. Although it was in the normal range, it was lower than that of the unmedicated group, 12.2 ng/ml. Further, the percentage of subjects who had very low SFC (below 3.0 ng/ml) in the group taking antibiotics was about twice that in the group not taking antibiotics.

The low SFC among antibiotic users may have been caused either by long term low folacin intake or by the effects of antibiotics on the folacin assay organism (3, 31). The folacin intakes of the girls taking antibiotics are shown with SFC (Table IV). Based on the low intakes of subjects 23 and 146 and their use of antibiotics, the low SFC would be expected, but their SFC were normal. Only one 24-hour food intake recall could be obtained for subject 23. Subject 93 reported marginal-

to-adequate folacin intake, but her SFC was low, possibly due to antibiotics. Similarly, folacin for subject 102 was above the RDA but SFC was below the overall mean. Low SFC of subjects 5, 47, 54, and 142 might have been due to low folacin intake (less than 250 μg), or antibiotics, or both. Because of possible effects of antibiotics, subsequent analyses do not include the data for antibiotic users.

TABLE III

DISTRIBUTIONS OF FOLACIN STATUS BY SERUM FOLACIN CATEGORIES

Serum Folacin Range (ng/ml)	All Subjects		No Antibiotics		Antibiotics	
	n	percent	n	percent	n	percent
<3.0	26	22.8	23	17.0	3	37.5
3.0-6.0	27	23.7	25	18.4	2	25.0
≥6.0	91	53.5	88	64.7	3	37.5
Total	144	100.0	136	100.0	8	100.0

TABLE IV

FOLACIN INTAKES AND SERUM FOLACIN CONCENTRATIONS (SFC) OF GIRLS TAKING ANTIBIOTICS

Subject No.	Folacin Intake (μg)	SFC (ng/ml)
5	230	2.8
23	30	11.6
47	115	0.8
54	80	5.2
93	368	5.1
102	413	6.9
142	105	0.6
146	190	20.2

Thirteen girls had the SFC higher than 30.0 ng/ml. Those girls might have had vitamin B_{12} deficiency, since vitamin B_{12} deficiency has increased SFC (86, 87). The intakes of folacin and vitamin B_{12} and SFC for those girls are presented in Table V. Of the 13 girls who had SFC above 30.0 ng/ml, 6 ingested less than 250 µg for folacin, but all of them except two had intakes of vitamin B_{12} of more than 3.0 µg, the RDA. Subject 86 consumed 97% of the RDA for vitamin B_{12}, but subject 108 got only 43% of the RDA. The high SFC of subject 108 might have been due to the low intake of vitamin B_{12}. The remaining 7 girls had marginal-to-adequate folacin intakes and all of them also had vitamin B_{12} above the RDA. Thus there was no clear evidence that the high SFC was caused by the lack of vitamin B_{12} in this study.

TABLE V

INTAKES OF FOLACIN AND VITAMIN B_{12} OF GIRLS WHO HAD SFC HIGHER THAN 30.0 ng/ml

Subject No.	SFC (ng/ml)	Folacin (µg)	Vitamin B_{12} (µg)
17	38.3	118*	3.27
38	36.4	189*	3.36
41	47.7	80*	16.65
86	31.8	243*	2.91*
108	39.1	95*	1.32*
167	30.1	210*	3.08
6	50.0	407	4.75
22	50.0	304	5.85
40	58.5	310	3.11
44	68.4	831	7.11
51	36.4	387	7.52
55	32.4	389	10.26
100	40.2	413	7.02

*Less than 250 µg for folacin or the RDA for vitamin B_{12}.

Based on the SFC, folacin deficiency was very common in adolescent girls. Only about half of the girls had normal SFC (equal to or above 6.0 ng/ml), and about one out of five was deficient (below 3.0 ng/ml). If the guideline of 2 ng/ml of one earlier study (25) was employed to interpret the SFC data of this study, 17 girls (12.5%) would still be considered to be deficient, a greater proportion than the 4.7% observed in that study. However, a different method for folacin assay was apparently used and the range of plasma folacin concentration in that study tended to be narrower than that of SFC in this study.

The method employed for the serum assay in this study was simpler than other methods (31-36), since it did not involve the autoclaving of serum samples, analysis of multiple dilutions of serum, or making buffer solution rather than using the commercial buffered media. The autoclaving of serum might destroy some serum folacin, since folacin is heat labile (36, 46). The storage of serum samples for about 15 months with ascorbic acid in this study did not seem to cause the loss of L. casei activity. This was consistent with the reports that folacin was stable if serum was stored at -20°C with that reducing agent (33, 90, 121).

Relationship of Folacin Intake and Serum Folacin

Dietary folacin was significantly correlated with SFC ($r = +0.25$, $p < .01$). However, a chi-square test did not show an association of folacin adequacy evaluated by the RDA and the folacin deficiency assessed by SFC (Table VI). Whether hypothesis 1, which stated,"There will be no significant relationship between dietary folacin and SFC," was accepted or rejected was dependent upon the approach used to test it. It could be rejected when a correlation coefficient was employed. On

the contrary, it could not be rejected if only the chi-square test was used. Since there was evidence from the former to contradict the null hypothesis 1, this hypothesis was rejected.

TABLE VI

FREQUENCIES OF FOLACIN ADEQUACY EVALUATED BY RDA AND FOLACIN DEFICIENCY ASSESSED BY SFC*

Folacin Intake (ug)	Serum Folacin (ng/ml)							
	<3.0		3.0-6.0		≥6.0		Total	
	n	percent	n	percent	n	percent	N	percent
<1/3 RDA	8	5.9	11	8.1	30	22.1	49	36.0
1/3-2/3 RDA	10	7.4	6	4.4	29	21.3	45	39.1
≥2/3 RDA	5	3.7	9	6.6	28	20.6	42	30.9
Total	23	16.9	26	19.1	87	64.0		

*$Chi^2 = 2.68$, $p = 0.613$.

Some girls had low folacin intakes but normal SFC, and some other girls had marginal-to-adequate folacin intakes but folacin deficiency as assessed by SFC. Serum folacin determination probably provided a more sensitive evaluation of the folacin nutritional status than dietary folacin because of the error inherent in collecting quantitative food intake data; and the availability of folacin from the diet was unknown. SFC is thought to depend on liver folacin stores (136). If liver stores were adequate, folacin would be released to the circulation

and be transported to the tissues for utilization when the dietary folacin was inadequate or unavailable. If liver stores were not adequate, due to long-term low folacin intake (117), newly absorbed folacin from the diet would be taken up rapidly by the tissues without greatly increasing SFC (119).

Relationships of Folacin Intake and Serum Folacin to Other Nutrients, Energy Intake and Hemoglobin

Table VII presents the correlation of folacin intake and of SFC with other nutrients, including vitamin B_{12}, vitamin C, iron, riboflavin, sucrose, and energy, and with HB concentration. There were positive correlations between the intake of folacin and intakes of vitamin B_{12}, vitamin C, iron, sucrose, and energy. SFC was correlated with dietary vitamin B_{12} and riboflavin as well as folacin. Therefore, hypothesis 2, that stated, "There will be no significant relationship between intakes of selected nutrient or energy and dietary folacin and SFC," was partially rejected.

Hemoglobin, which was greater than 11.5 g/dl for all girls, was not correlated with the folacin intake or SFC. So, hypothesis 3, which stated, "There will be no significant relationship between HB concentration and dietary folacin or SFC," was accepted for this group of non-anemic girls.

Relationships among Folacin, Iron Status, and Low Hemoglobin

For the sample as a whole, 8 girls had the HB concentrations below 12.0 g/dl. Low HB may be caused by an iron deficiency or

TABLE VII

CORRELATION COEFFICIENTS FOR DIETARY FOLACIN, SERUM FOLACIN, OTHER NUTRIENTS, ENERGY, AND HEMOGLOBIN CONCENTRATION[+]

	Folacin	Vitamin B_{12}	Vitamin C	Iron	Zinc	Riboflavin	Methionine	Sucrose	Energy	Hemoglobin
Folacin	--	+0.469**	+0.329**	+0.510**	+0.108	+0.147	+0.054	+0.196*	+0.351**	+0.006
SFC	+0.247**	+0.252**	+0.164	+0.132	+0.158	+0.276**	+0.038	-0.067	+0.044	+0.080

*$p < 0.05$.

**$p < 0.01$.

[+]N = 150 except N = 144 for HB concentrations and N = 136 for SFC.

folacin deficiency, or both (95, 96), or by a lack of other nutrients such as riboflavin (108) or by the other abnormal conditions. Girls with the lowest HB values were examined on the basis of SFC and serum ferritin (Table VIII), because these biochemical tests were thought to be more sensitive and accurate measurements of the nutritional status than the dietary data (35).

TABLE VIII

SFC, SERUM FERRITIN AND RIBOFLAVIN INTAKES OF GIRLS WITH LOWEST HB CONCENTRATION

Subject No.	HB Concentration (g/dl)	SFC (ng/ml)	Serum Ferritin (ng/ml)	Dietary Riboflavin (mg)
32	11.48	4.7*	3*	2.33
53	11.65	2.6*	2*	1.65
142	11.67	0.7*	13*	1.68
148	11.64	2.6*	18	0.80*
104	11.57	6.8	11*	1.62
120	11.94	9.8	10*	1.15
39	11.94	9.6	63	1.87
85	11.90	10.0	26	1.11
N = 8 Mean	11.77	6.0	18	1.53

*Deficient, low, or intake less than 2/3 RDA.

The low HB concentrations for subjects 32, 53, 142, and 148 might have been caused by either folacin or iron deficiency or both. All of them had the serum ferritin below 15 ng/ml, which indicated low iron stores (137), and low SFC. The low intake of riboflavin (less than 2/3 the RDA) for subject 148 might contribute to her low HB in addition to

the folacin deficiency. Subjects 104 and 120 had normal SFC but low serum ferritin. Their low HB concentrations might indicate the iron deficiency. Subjects 39 and 85 had normal SFC and serum ferritin and exceeded 2/3 the RDA for riboflavin. The cause might be due to the race (see Appendix B), since the black tends to have lower HB than the white (13, 21, 23).

The Association of Mean Corpuscular Volume (MCV) with SFC, and Vitamin B_{12} Intake

Twenty out of 150 girls had MCV above 85.0 fl, and thus were near or at the upper limit of the normal range for red cell volume. This suggested folacin or vitamin B_{12} deficiency, or both, since either condition has resulted in megaloblastic blood cells (11, 84). In contrast, iron deficiency has decreased MCV to below normal (11). Table IX reports the SFC and dietary vitamin B_{12} of those girls with the MCV above 85.0 fl.

Of 20 girls with MCV above 85.0 fl, 3 had both low SFC (2 were deficient) and low vitamin B_{12} intake, 4 had low SFC (3 were deficient) and adequate vitamin B_{12}, 8 had normal SFC and inadequate vitamin B_{12}, and 5 had both normal SFC and adequate vitamin B_{12}. Of the rest of the girls, with MCV below 85.0 fl, 21 had both low SFC (8 were deficient) and inadequate vitamin B_{12}, 21 had low SFC (9 were deficient) with adequate dietary vitamin B_{12}, 27 had normal SFC without adequacy of vitamin B_{12}, and 47 had both normal SFC and adequate vitamin B_{12} from the diet. Thus, 5 out of 20 girls (25%) with the MCV above 85.0 fl were folacin deficient, whereas only 17 out of 116 girls (15%) with MCV below 85.0 fl were folacin deficient; 11 of 20 girls (55%) with MCV above 85.0 fl and only 48 of 116 girls (41%) with MCV below 85.0 fl did not meet

TABLE IX

SFC AND DIETARY VITAMIN B_{12} OF SUBJECTS WITH MCV ABOVE 85.0 fl

Subject No.	MCV (fl)	SFC (ng/ml)	Dietary Vitamin B_{12} (µg)
67	87.0	0.7*	1.05*
97	96.0	0.5*	2.11*
163	85.4	3.1*	2.41*
29	85.8	2.8*	4.74
42	87.4	1.5*	3.01
77	92.2	4.0*	3.87
121	91.2	0.7*	3.65
66	86.3	8.7	2.38*
82	91.4	17.0	2.80*
142	86.1	16.4	1.95*
152	85.7	6.6	0.91*
162	85.4	7.9	2.31*
168	87.2	25.2	2.61*
169	85.6	12.2	2.69*
174	92.0	6.9	1.31*
19	86.5	9.0	3.35
84	90.5	10.1	5.75
91	85.4	16.0	3.50
94	89.2	11.1	3.50
167	86.5	30.1	3.08
N = 20 Mean	88.0	10.0	2.85

*Low or deficient status or intake below the RDA

the RDA for vitamin B_{12}. Also, a higher proportion of the girls with MCV below 85.0 fl had both normal SFC and adequate dietary vitamin B_{12} than that of the girls with MCV above 85.0 fl (40% versus 25%). These data suggested that folacin deficiency and vitamin B_{12} inadequacy might have increased the MCV of some girls in this study.

Folacin Intake as Affected by Selected Independent Variables

In Table X, folacin intake was related to several variables other than the nutrients already discussed. Each variable was tested by itself as a class variable in a general linear model (GLM), and the least squares means (LSM) are presented. Of 11 independent variables, only three, place of residence, body weight, and the use of nutritional supplements, were related to folacin intake. These three factors were then tested together in two models in which body weight was treated as a continuous or as a class variable. The R-square value is a measure of the fraction of the variance in the dependent variable (dietary folacin) explained by the regression equation. The R-square was 0.271 when weight was treated as a continuous variate, and 0.307 when tested as a class variable. Thus 27.1-30.7% of the variance in different models was explained by those three independent variables with the use of nutritional supplements accounting for the most variance.

Relationships of Dietary Folacin to Place of Residence, Body Weight and Use of Nutritional Supplements

All of the girls were categorized according to the place of residence, city, town, or rural area. The folacin intakes of the girls in

TABLE X

FOLACIN INTAKES ACCORDING TO INDEPENDENT VARIABLES

Independent Variables		Folacin Intake (µg)											
		LSF*	PR>F	< 1/3 RDA		1/3-2/3 RDA		≥ 2/3 RDA		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Race	White	223	.206	42	28.0	34	22.7	39	26.0	115	76.7	4.05	.132
	Black	188		14	9.3	15	10.0	6	4.0	35	23.3		
Age (years)	13.5	239	.250	18	12.1	17	11.4	21	14.1	56	46.7	5.54	.236
	13.5-15.5	203		22	14.8	24	16.1	15	10.1	61	10.9		
	15.5	193		16	10.7	7	4.7	9	6.0	32	21.4		
Place of Residence	City	219[a]	.001**	21	14.0	20	13.3	17	11.3	58	38.7	12.42	.014
	Town	247[a]		15	10.0	17	11.3	24	16.0	56	37.3		
	Rural	156[b]		20	13.3	12	8.0	4	2.7	36	24.0		

TABLE X (Continued)

Independent Variables		Folacin Intakes (µg)											
		LSM*	PR>F	<1/3 RDA		1/3-2/3 RDA		≥2/3 RDA		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Height (cm)	156.2	250		15	10.3	12	8.2	18	12.3	45	30.8		
	156.2-161.3	203	.148	22	15.1	14	9.6	13	8.9	49	33.6	5.58	.233
	161.3	197		17	11.6	22	15.1	13	8.9	52	35.6		
Weight (kg)	46.2	260[a]	**	17	11.6	12	8.2	21	14.4	50	34.2		
	46.2-54.9	190[b]	.027	19	13.0	15	10.3	12	8.2	46	31.5	5.76	.218
	54.9	196[b]		19	13.0	20	13.7	11	7.5	50	34.2		
Per Capita Income ($ yearly)	2700	195		9	6.1	9	6.1	4	2.7	22	15.0		
	2700-5700	213	.773	20	13.6	17	11.6	16	10.9	53	36.0	2.08	.722
	5700	220		27	18.4	21	14.3	24	16.3	72	49.0		

TABLE X (Continued)

Independent Variables		Folacin Intakes (µg)											
		LSM*	PR>F	<1/3 RDA		1/3-2/3 RDA		≥2/3 RDA		Total		Chi²	p
				n	percent	n	percent	n	percent	N	percent		
Menstrual Status	post-menarcheal	202	.082	40	27.4	34	23.3	27	18.5	101	69.2	1.93	.381
	pre-menarcheal	243		14	9.6	14	9.6	17	11.6	45	30.8		
Nutritional Supplements	use	408	.001	0	0.0	1	0.7	14	9.3	15	10.0	31.96	.001
	non-use	193		56	37.3	48	32.0	31	20.7	135	90.0		
Dieting Practice	gaining weight	291	.082	2	1.4	2	1.4	3	2.1	7	4.9	2.32	.313
	losing weight	188		26	18.0	21	14.6	14	9.7	61	42.4		
	neither	229		25	17.4	25	17.4	26	18.0	76	52.8		

TABLE X (Continued)

Independent Variables		Folacin Intakes (µg)											
		LSM*	PR>F	<1/3 RDA		1/3-2/3 RDA		≥2/3 RDA		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Alcohol	use	225	.454	26	17.8	20	13.7	24	16.4	70	48.0	1.53	.466
	non-use	208		28	19.2	28	19.2	20	13.7	76	52.0		
Tobacco	use	170	.235	4	2.8	6	4.2	2	1.4	12	8.3	2.08‡	.354
	non-use	221		49	34.0	41	28.5	42	29.2	132	91.7		

*The least squares mean.

+Chi-square test only for the groups of losing weight and neither gaining nor losing weight.

‡Chi-square test not valid due to the small size of subjects in the categories.

**Means sharing the same superscript are not different ($p > .05$).

towns, 247 µg, and in the city, 219 µg, exceeded that of the rural girls, 156 µg (Table X). However, there was no significant difference of the folacin intake between the girls in the city and in towns. A chi-square test indicated an association of the place of residence with the categories of folacin adequacy as evaluated by the RDA.

Girls were divided into three groups according to their weight (Table X). The girls of the lightest group consumed more folacin (260 µg) than the other two heavier groups (190 and 196 µg). The chi-square test did not show that folacin adequacy evaluated by the RDA was associated with the weight categories significantly.

Only 15 girls took nutritional supplements which contained folacin; the other girls took either no supplement or one which did not contain folacin (Table X). The folacin intake for the girls taking folacin nutritional supplements, 408 µg, was much higher than that for girls not taking folacin supplements, 193 µg. Both GLM and chi-square tests showed a significant relationship of the folacin intake to the use or non-use of a nutritional supplement. The amount of folacin in the nutritional supplements taken by the girls was 300 or 400 µg depending on the brands. All girls taking nutritional supplements were from the middle or high income families in this study. This was the same as another study (25).

Dietary Folacin and Race, Age, Height, Per Capita Income, Menstrual Status, Dieting Practice, Alcohol Consumption, and Use of Tobacco

All subjects were divided into the groupings indicated in Table X according to categories of responses or ranges of values for the continu-

ous variables, selected to give groups of similar size. Black girls have sometimes consumed more folacin than white girls (25), but not in this study (Table X). Race was not a significant factor affecting the folacin intake whether examined in the analysis of variance as a linear factor or by the chi-square test of frequencies (Table X). Neither age nor income affected the folacin intake, which was consistent with another study (25). Sexual maturity was a factor which did influence the folacin intake (25); mature girls consumed more folacin than immature ones. However, that was not confimed by this study, where sexual maturity was based simply on the menstrual status, pre-menarcheal or post-menarcheal. Other variables, height, dieting practice, alcohol consumption, and use of tobacco, did not affect the folacin intake.

Only one question about the dieting practice of the girls interviewed on the blood-drawing day was examined in this study. That was "Are you presently trying to gain or lose weight, or neither?" (see Appendix A - Form D1). Of 144 girls, 7 reported they were trying to gain weight, 61 were trying to lose weight, and 76 were not attempting either (Table X).

Two variables, alcohol consumption and the use of tobacco, were selected in this study because of the high incidence of drinking and smoking reported among teenagers (138). Folacin deficiency is often found in alcoholic populations (68, 111). Whether smoking affects the folacin nutritional status is unknown, since no research has been done in that area. In this study, drinking and smoking were not reported so often as for the participants of another study (138). Less than half of the girls admitted drinking alcohol and most of them reported doing so rarely. Only 8.3% of the girls reported using tobacco (Table X).

Serum Folacin as Affected by Selected Independent Variables

Table XI presents the LSM for serum folacin and distributions of SFC according to the categories of 11 independent variables. The chi-square values are also reported. Among those variables examined individually in the GLM, only age and menstrual status were related to SFC significantly. Those two variables were also tested together in the GLM. The R-square was 0.04 with neither the age ($p = 0.592$) nor menstrual status category ($p = 0.156$) significantly related to the SFC after adjusting for the effect of the other.

Relationships of SFC to Age and Menstrual Status

The LSM of SFC for the girls of less than 13.5, between 13.5 and 15.5, and older than 15.5 years were 16.0, 9.4, and 11.2 ng/ml respectively. F test showed the SFC of the youngest age group was higher than that of the other older groups (Table XI). However, the SFC of the two older groups were not different from each other. On the other hand, a chi-square test did not show an association of folacin deficiency, as assessed by the SFC, with age category. Another study (25) did not show that age affected the plasma folacin concentration.

The pre-menarcheal girls had higher SFC than the post-menarcheal girls (Table XI), whether based on an F or chi-square test. This indicated that the more mature girls tended to have lower SFC and confirmed the result of another study (25). The cycling uterus with its alternating periods of cell devision and rest is controlled by the sex hormones, estrogens and progestogens. The folacin conjugase activity of the uterus of rats increased during proestrus, the time of maximal

TABLE XI

SFC ACCORDING TO INDEPENDENT VARIABLES

Independent Variables		Serum Folacin Concentration (SFC) (ng/ml)											
		LSM*	PR>F	<3.0		3.0-6.0		≥6.0		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Race	White	12.6	.420	16	11.8	22	16.2	68	50.0	106	77.9	1.62	.446
	Black	10.5		7	5.2	4	2.9	19	14.0	30	22.1		
Age (years)	13.5	16.0[a]**	.022	3	2.2	10	7.4	36	26.5	49	36.0	6.68	.154
	13.5-15.5	9.4[b]		14	10.3	10	7.4	34	25.0	58	42.6		
	15.5	11.2[b]		6	4.4	6	4.4	17	12.5	29	21.3		
Place of Residence	City	10.1	.318	9	6.6	10	7.4	33	24.3	52	38.2	2.05	.726
	Town	13.3		9	6.6	7	5.2	34	25.0	50	36.8		
	Rural	13.6		5	3.7	9	6.6	20	14.7	34	25.0		

TABLE XI (Continued)

Independent Variables		Serum Folacin Concentration (SFC) (ng/ml)											
		LSM*	PR>F	<3.0		3.0-6.0		≥6.0		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Height (cm)	156.2	13.7		2	1.5	9	6.6	30	22.1	41	30.2		
	156.2-161.3	13.3	.276	7	5.2	6	4.4	32	23.5	45	33.1	10.80	.029
	161.3	10.1		14	10.3	11	8.1	25	18.4	50	36.8		
Weight (kg)	46.2	12.2		4	2.9	10	7.4	30	22.1	44	32.4		
	46.2-54.9	14.2	.306	3	2.2	9	6.6	32	23.5	44	32.4	14.46	.006
	54.9	10.2		16	11.8	7	5.2	25	18.4	48	35.3		
Per Capita Income ($ yearly)	2700	14.8		4	3.0	0	0.0	16	11.8	20	14.8		
	2700-5700	13.2	.330	6	4.4	11	8.2	33	24.4	50	37.0	6.66	.155
	5700	10.6		13	9.6	14	10.4	38	28.2	65	48.2		

TABLE XI (Continued)

Independent Variables		Serum Folacin Concentration (SFC) (ng/ml)											
		LSM*	PR>F	<3.0		3.0-6.0		≥6.0		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Menstrual Status	post-menarcheal	10.5[a]	.018	21	15.4	18	13.2	54	39.7	93	68.4	7.20	.027
	pre menarcheal	15.9[b]		2	1.5	8	5.9	33	14.3	43	31.6		
Nutritional Supplements	use	16.1	.190	3	2.2	1	0.7	11	8.1	15	11.0	1.69[+]	.429
	non-use	11.7		20	14.7	25	18.4	76	55.9	121	99.0		
Dieting	gaining weight	10.9	.916	2	1.5	0	0.0	4	3.0	6	4.4	1.85[‡]	.396
	losing weight	11.9		12	8.9	9	6.7	39	28.9	60	44.4		
	neither	12.6		10	7.4	17	12.6	42	31.1	69	51.1		

TABLE XI (Continued)

Independent Variables		Serum Folacin Concentration(SFC) (ng/ml)											
		LSM*	PR>F	<3.0		3.0-6.0		≥6.0		Total		Chi^2	p
				n	percent	n	percent	n	percent	N	percent		
Alcohol	use	13.8	.127	11	8.1	11	8.1	44	32.4	66	48.5	0.55	.758
	non-use	10.6		12	8.8	15	11.0	43	31.6	70	51.5		
Tobacco	use	8.1	.235	2	1.5	3	2.2	7	5.2	12	9.0	2.08+	.354
	non-use	12.6		21	15.7	23	17.2	78	58.2	122	91.0		

*The least square mean.

+Chi-sqiare test not valid due to the small size of subjects in the categories.

‡Chi-square test only for the groups of losing weight and neither gaining nor losing weight.

**Measn sharing the same superscript are not different ($p > .05$).

estrogen secretion. It was greater than those observed at any other stage in the cycle. Folacin and conjugase might participate in the regulation of cell proliferation and rest during the cycle (6). Sex hormones appear to act on the target organs via the regulation of protein synthesis (139), and folacin coenzymes function as important one-carbon donors in amino acid and DNA synthesis, also necessary for tissue growth (3-7, 82). Sex hormones control the utilization of folacin in the uterus. In the pre-menarcheal state, progestogens tend to inhibit the cell multiplication in the endometrium, the lining of the uterus. In the post-menarcheal state, folacin is essential to the action of estrogens in promoting tissue proliferation in the uterus. The higher SFC in pre-menarcheal girls than in post-menarcheal girls is possible because less folacin is required to saturate the tissues of a small body in pre-menarcheal state, and it may require more folacin to saturate the tissues under estrogenic stimulus in post-menarcheal state. On the other hand, the sex hormones might affect the absorption of the polyglutamate forms of folacin during the cycle, according to the study of the absorption of folacin in women who were on oral contraceptives (140), and result in the different SFC between pre-menarcheal and post-menarcheal states.

SFC and Race, Age, Place of Residence, Height, Weight, Per Capita Income, Use of Nutritional Supplements, Alcohol Consumption and Use of Tobacco

Neither the F nor T test showed a significant difference in the SFC within any of the categories of race, age, place of residence, height, weight, per capita income, use of nutritional supplements,

dieting practice, alcohol consumption, or use of tobacco (Table XI); nor did a chi-square test indicate an association of folacin deficiency with any of those variables except height and weight. The lack of any relationship of income and use of nutritional supplements to the SFC did not confirm the result of another study (25). In that study, the girls of higher income families had higher plasma folacin than the girls of lower income due to taking folacin-containing supplements.

In this study, the girls in the tallest group and those in the heaviest group had the highest incidences of folacin deficiency (Table XI). The reasons for that might be low ingestion of folacin, at least by heavy girls (Table X), and a greater requirement for taller and heavier girls than for shorter and lighter girls to meet the body needs.

The use of alcohol did not affect the SFC in this study. In general, alcohol ingestion has reduced the absorption and circulation of folacin (111). The small amounts of alcohol reported by most users may have been enough to prevent a fall in serum folacin. Of 66 girls who reported drinking alcohol, 41 did so rarely, 12 did once per month, 3 did so twice monthly, and only 8 girls admitted drinking alcohol one to two times per week (see Appendix B).

Based on the results above, Hypothesis 4, which stated, "There will be no significant relationship of race, age, place of residence, height, weight, per capita income, menstrual status, use of nutritional supplements, dieting practice, alcohol sonsumption, and use of tobacco to dietary folacin and SFC," was partially rejected.

CHAPTER V

SUMMARY AND CONCLUSIONS

The purpose of this study was to investigate the folacin nutritional status in adolescent girls. The serum folacin was assayed by a microbiological method, and dietary folacin and other nutrients were calculated from food intake recalls. The average of two 24-hour recalls was taken. Questionnaires and interviews with subjects and their mothers provided the information necessary to this study. The independent variables included race, age, place of residence, height, weight, per capita income, menstrual status, use of nutritional supplements, dieting practice, alcohol consumption, and use of tobacco. The relationships between dietary folacin, SFC, and independent variables were identified by the statistical methods, including the Pearson Moment Product Correlation Coefficient, analysis of variance with T or F test to compare means, and the chi-square test of distributions among the categories. Subjects taking antibiotics or having SFC above 30.0 ng/ml, HB concentrations below 12.0 g/dl, or MCV above 85.0 fl were examined in relation to the folacin status, dietary vitamin B_{12}, dietary riboflavin, or serum ferritin.

The following findings resulted from this study:

1. The folacin intakes of many adolescent girls were low when compared with the RDA. The mean folacin intake of 150 girls was only 53.8% RDA, and only about one-third of the girls had folacin intakes

above 2/3 the RDA.

2. Folacin deficiency, which was assessed by the serum folacin assay, was common in adolescent girls. Only about half of the girls had normal SFC (above or equal to 6.0 ng/ml), according to the WHO and the Ten-State Nutrition Survey guidelines. About one out of five girls was deficient in folacin (SFC below 3.0 ng/ml).

3. Serum folacin increased with increasing dietary folacin.

4. Folacin intake was correlated with dietary vitamin B_{12}, vitamin C, iron, sucrose, and energy, but not with dietary zinc, riboflavin, or methionine.

5. Serum folacin was correlated with dietary vitamin B_{12} and riboflavin, but not with other nutrients or energy.

6. Only one out of 20 girls with high values of SFC might have been caused by vitamin B_{12} deficiency.

7. Based on SFC and serum ferritin measurements, 4 out of 8 girls with low HB concentration might have been deficient in both folacin and iron. No one of them was deficient in folacin only.

8. Folacin deficiency and vitamin B_{12} inadequacy might have increased the MCV of some girls.

9. Among 11 independent variables examined individually, only three were significantly related to dietary folacin. The girls living in the rural areas had lower folacin intakes than the girls living in the city or in towns. The girls weighing less than 46.2 kg consumed more folacin than the girls of two heavier weight groups. The use of nutritional supplements greatly increased the folacin intake.

10. Among 11 independent variables, only two were significantly related to the SFC. Girls younger than 13.5 years had higher SFC than

girls of two older groups. The pre-menarcheal girls had higher SFC than the post-menarcheal girls, when only that variable was considered. When adjusted for age, however, that difference disappeared.

11. The associations of adequate dietary folacin, defined as meeting 2/3 the RDA, with place of residence and use of a nutritional supplement were significant. But the associations of folacin adequacy based on serum assay with those two variables were not significant.

12. Folacin adequacy assessed by SFC was associated with height, weight, and menstrual status, but the associations of the adequacy of folacin intake relative to the RDA with those three independent variables were not significant.

REFERENCES

(1) Robison, F. A.: The Vitamin Co-Factors of Enzyme System. N.Y.: Pergamon Press Ltd, 1966.

(2) Rechcigl, M. Jr.: CRS Handbook Series in Nutrition and Food Section A: Nutrition Disorders. Vol.II. West Palm Beach: CRS Press, Inc., 1978.

(3) Blakley, R. L.: The Biochemistry of Folic Acid and Related Pteridines. Amsterdam, London. North-Holland Publishing Co., 1969.

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

(5) Goodhart, R. S., and Shils, M. E.: Modern Nutrition in Health and Disease. Dietotherapy. 6th ed. Philadephia: Lea & Febiger, 1980.

(6) The National Research Council: Folic Acid. Biochemistry and Physiology in Relation to the Human Requirement. Washington, D.C.: National Academy of Science, 1977.

(7) The Nutrition Fundation: Nutrition Reviews. Present Knowledge in Nutrition. 4th ed. New York, 1976.

(8) Halsted, C. H.: Folate deficiency and small intestine. In Botes, M. I., and Reynolds, E. H., eds.: Neurology, Psychiatry and Internal Medicine. New York: Raven Press, 1979.

(9) Halsted, C. H.: Intestinal absorption and malabsorption of folate. Ann. Rev. Med. 31:79-87, 1980.

(10) Bethell, F. H., Meyers, M. C., Andrews, G. A., Mich, A. A., Swendseid, M. E., Bird, O. D., and Brown, R. A.: Metabolic function of pteroylglutamic acid and its hexaglutamyl conjugate. I. Hematologic and urinary excretion studies on patients with macrocytic anemia. J. Lab. Clin. Med. 32:3-22, 1947.

(11) Clarkson, D. R., and Moore, E. M.: Reticulocyte size in nutritional anemia. Blood 48:669-677, 1976.

(12) WHO Scientific Group: Nutritional Anemias. Wld Hlth Org. Techn. Rep. Ser. No. 405, Geneva, Switzerland. World Health Organization, 1968.

(13) Ten-State Nutrition Survey (1968-1970): U.S. Health Services and Mental Health Administration. Washington D.C. DHEW Pub. No. (HSM)72-8130, 1972.

(14) Thomas, J. A., and Call, D. L.: Eating between meals, a nutri-problem among teens? Nutr. Rev. 31:137-139, 1973.

(15) Kelly, J. T., Patten, S. E., and Johannes, A.: Analysis of self-reported eating and related behaviors in an adolescent population. Nutr. Res. 2:417-432, 1982.

(16) NCHS: Preliminary Findings of the First Health and Nutrition Examination Survey. United States, 1971-1972: Dietary Intake and Biochemical Findings. DHEW Pub. No.(HRA)74-1219-1, U.S. Dept. Health, Education, and Welfare. Washington D.C. 1974.

(17) Hinton, M. A., Eppright, E. S., Chadderdon, H., and Wolins, L.: Eating behavior and dietary intake of girls 12 to 14 years old. J. Am. Dietet. A. 43: 223-227, 1963.

(18) Hodges, R. E., and Krehl, W. A.: Nutritional status of teenagers in Iowa. Am. J. Clin. Nutr. 17:200-210, 1965.

(19) Oklahoma State Dept. Edu.: Nutrition Education. A Needs Assessment for Oklahoma. 1978 report, Oklahoma State University. 1981.

(20) Hurley, L. S.: Developmental Nutrition. Englewood Cliffs, New : Prentice-Hall, Inc., 1980.

(21) Daniel, W. A. Jr., Gaines, E. G., and Bennett, E. L.: Iron intake and transferrin saturation in adolescents. J. Pediatr. 86:288-292, 1975.

(22) Greenwood, C. T., and Richardson, D. P.: Nutrition during adolescence. World Rev. Nutr. Diet. 33:1-41, 1979.

(23) Voors, A. W., Frank, G. C., Srinivasan, S. R., Webber, L. S., and Berenson, G. S.: Hemoglobin levels & dietary iron in puberscent children in a biracial community. Public Health Reports. 96:45-49, 1981.

(24) Bureau of Com. Hlth. Serv.: Iron Nutrition in Adolescence. 10 pages publication. DHEW Pub. No.(HSA)77-5100, Hlth. Serv. Adm. Rockville, MD. Public Hith. Serv. DHEW.

(25) Daniel, W. A. Jr., Gaines, E. G., and Bennett, E. L: Dietary intakes and plasma concentrations of folate in health adolescents Am. J. Clin. Nutr. 28:363-370, 1975.

(26) Mark, M. S. V., and Wright, A. C.: Hemoglobin and folate levels of pregnant teenagers. J. Am. Dietet. A. 61:511-516, 1972.

(27) Daniel, W. A. Jr., Mounger, J. R., and Perkins, J. C.: Obstetric and fetal complications in folate-deficient adolescent girls. Am. J. Obstet. Gynecol. 111:233-238, 1971.

(28) Food & Nutr. Bd.: Recommended Dietary Allowances. 9th ed. Washington, D.C.: Natl. Acad. Sci., 1980.

(29) Perloff, B. P., and Butrum, R. R.: Folacin in selected foods. J. Am. Dietet. A. 70:161-172, 1977.

(30) Baker, H., Herbert, V., Frank, O., Pasher, I., Hunter, S. H., Wasserman, L. R., and Sobotka, H.: A microbiological method for detecting folic acid deficiency in man. Clin. Chem. 5:275-280, 1959.

(31) Herbert, V.: Aseptic addition method for Lactobacillus casei assay of folate activity in human serum. J. Clin. Path. 19:12-16, 1966.

(32) Spray, C. H.: Microbiological assay of folic acid activity in human serum. J. Clin. Path. 17:660-665, 1964.

(33) Herbert, V., Fisher, R., and Koontz, B. J.: The assay and nature of folic acid activity in human serum. J. Clin. Invest. 40:81-91, 1961.

(34) Cooperman, J. M.: Microbiological assay of serum and whole-blood folic acid activity. Am. J. Clin. Nutr. 20:1015-1024, 1967.

(35) Sauberlich, H. E., Dowdy, R. P., and Skala, J. H.: Laboratory Tests for the Assessment of Nutritional Status. Critical Reviews in Clinical Laboratory Science. 4:263-274, 1973.

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

(37) Wills, L., and Evans, B. D. F.: Tropical macrocytic anemia: its relation to pernicious anemia. Lancet 2:416-421, 1938.

(38) IUNS Commettee on Nomenclature: Tentitve rules for generic descriptors and trivial names for vitamins and related compound. J. Nutr. 101:133-140, 1971.

(39) The Nutrition Fundation:Present Knowledge in Nutrition. 2nd ed. New York, 1965.

(40) Gyorgy, P., and Pearson, W. N.: The Vitamins. Vol.VIII. 2nd ed. New York and London, Academic Press, 1967.

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

(42) Mitchell, H. K., Snell, E. E., and Williams, R. J.: The concentration of "folic acid". J. Am. Chem. Soc. 63:2284, 1941.

(43) Angier, R. B., Boothe, J. H., and Hutchings, B. L.: Synthesis of a compound identical with the L. casei factor isolated form liver. Science 102:227-228, 1945.

(44) Jukes, T. H., and Stokstad, E. L. R.: Pteroylglutamic acid and related compounds. Physio. Rev. 28:51-106, 1948.

(45) Wagner, A. F., and Folkers, K.: Vitamins and Coenzymes. New York: Wiley (Interscience) Inc., 1964.

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

(47) Hurdle, A. D. F.: The assay of folate in food. Nutrition 27:12-16, 1973.

(48) Matoth, Y., Pinkas, A., and Sroka, C.: Studies on folic acid in infancy. III. Folates in breast fed infants and their mothers. Am. J. Cli. Nutr. 16:356-359, 1965.

(49) Stokstad, E. L. R., Fordham, D., and Grunigen, A.: The inactivation of pteroylglutamin acid (liver Lactobacillus casei factor) by light. J. Biol. Chem. 167:877-878, 1947.

(50) O'Broin, J. D., Temperley, I. J., Brown, J. P., and Scott, J. M.: Nutritional stability of various naturally occuring monoglutamate derivatives of folic acid. Am. J. Clin. Nutr. 28:438-444, 1975.

(51) Silverman, M., Keresztesy, J. C., Koval, G. J., and Gardiness, R. C.: Citrovorum factor and the synthesis of formylglutamic acid. J. Biol. Chem. 226:83-94, 1957.

(52) Bothe, B., Hultquist, M. E., Fahenback, M. J., Cosculick, C. B., Broquist, H. P., Brochman, J. A. J., Smith, J. M. Jr., Parker, R. P., Stokstad, E. L. R., and Jukes, T. H.: Synthesis of Leucovorin. J. Am. Chem. Soc. 74:3247-3252, 1952.

(53) Rabinowitz, J. C., and Pricer, W. E. Jr.: Formiminotetrahydrofolic acid and methenyltetrahydrofolic acid as intermediates in the formation of N^{10}-formyltetrahydrofolic acid. J. Am. Chem. Soc. 78:5702-5704, 1956.

(54) May, M., Bardos, T. J., Barger, F. L., Lansford, M., Ravel. J. M., Sutherland, G. L., and Shieve, W.: Synthetic and degradative investigations of the structure of folinic acid - SF. J. Am. Chem. Soc. 73:3067-3075, 1951.

(55) Stokstad, E. L. R., Hutchings, R. L., Mowat, J. H., Boothe, J. H., Waller, C. W., Angier, R. B., Semb, J., and SubbaRaw, Y.: The degradation of the fermentation Lactobacillus casei factor. J. Am. Chem. Soc. 70:5-9, 1948.

(56) Butterworth, C. E. Jr., Santini, R. Jr., and Frommeyer, W. B. Jr.: The pteroylglutamate components of American diets as determined by chromatographic fractionation. J. Clin. Invest. 42:1929-1939, 1963.

(57) Halsted, C. H.: The intestinal absorption of folates. Am. J. Clin. Nutr. 32:846-855, 1979.

(58) Butterworth, C. E. Jr., and Krumdieck, C. L.: Intestinal absorption of folic acid monoglutamates and polyglutamates: a brief review of some recent development. Brit. J. Haematol. 31(suppl):111-118, 1975.

(59) Halsted, C. H., Reisenauer, A., Back, C., and Gotterer, G. S.: In vitro uptake and metabolism of pteroylpolyglutamate by rat small intestine. J. Nutr. 106:485-492, 1976.

(60) Baker, H., Frank, O., Feingold, S., Ziffer, H., Gellene, R. A., Leevy, C. M., and Sobotka, H.: The fate of orally and pareterally administered folates. Am. J. Clin. Nutr. 17:88-95, 1965.

(61) Elsorg, L.: Folic acid: a new approach to the mechanism of its intestinal absorption. Danish Med. Bull. 21:1-11, 1974.

(62) Swenseid, M. E., Bird, O. D., Brown, R. A., and Bethell, F. H.: Metabolic function of pteroylglutamic acid and its hexaglutamyl conjugate. II. Urinary excretion studies on normal persons. Effect of a conjugase inhibitor. J. Lab. Clin. Med. 32:23-27, 1947.

(63) Bird, O. D., Robbins, M., Vandenbelt, J. M., and Pfiffner, J. J.: Observations on vitamin B_c conjugase from hog kidney. J. Biol. Chem. 163:649-659, 1946.

(64) Luther, L., Santini, R., Brewster, C., Perez-Santiaga, E., and Butterworth, C. E. Jr.: Folate binding by insoluble components of American and Purto Rican diet. Ala. J. Med. Sci. 2:389-393, 1965.

(65) Russell, R. M., Ismail-Beigi, F., and Reinhold, J. G.: Folate content of Iranian breads and the effect of their fiber content on the intestinal absorption of folic acid. Am. J. Clin. Nutr. 29: 799-802, 1976.

(66) Ristow, K. A., Gregory, J. F., and Damron, B. L.: Effects of dietary fiber on the bioavailability of folic acid monoglutamate. J. Nutr. 112:750-758, 1982.

(67) Gerson, C. D., Gohen. N., Hepner, G. W., Brown, N., Herbert, V., and Janowitz, H. D.: Folic acid absorption in man: enhancing effect of glucose. Gastroenterology 61:224-227, 1971.

(68) Halsted, C. H., Robles, E. A., and Mezey, E.: Intestinal malabsorption in folate-deficient alcoholics. Gastroenterology 64:526-532, 1973.

(69) Hepner, G. W., Booth, C. C., Cowan, J., Hoffbrand, A. V., and Mollin, D. L.: Absorption of crystalline folic acid in man. Lancet

2:302-306, 1968.

(70) Floch, M. H.: Recent contributions in intestinal absorption and malabsorption. A review. Am. J. Clin. Nutr. 22:327-351, 1969.

(71) Herbert, V.: Absorption of vitamin B_{12} and folic acid. Gastroenterology 54:110-115, 1968.

(72) Herbert, V., Larrabee, A. R., and Buchanan, J. M.: Studies on the identification of a folate compound of human serum. J. Clin. Invest. 41:1134-1138, 1962.

(73) Zettner, A., and Duly, P. E.: Separation of folate binding protein from human serum by DEAE-cellulase column chromatography. Clin. Chem. 22:1047-1052, 1976.

(74) Spector, R., Lorenzo, A. V., Drum, D. V.: Serum binding of methyltetrahydrofolic acid. Biochem. Pharmacol. 24:542-544, 1975.

(75) Neal, G. E., and Williams, D. C.: The fate of intravenously injected folate in rats. Biochem. Pharmocol. 14:903-914, 1965.

(76) Herbert, V.: Predicting nutrient deficiency by formula. New Engl. J. Med. 284:976-977, 1971.

(77) Michael, W.: Polygammaglutamyl metabolites of folic acid in human liver. Lancet 1:743-745, 1973.

(78) Korevaar, W. C., Geyer, M. A., Knapp, S., Hsu, L. L., and Mandell, A. J.: Regional diestribution of 5-methyltetrahydrofolic acid in brain. Nature New Biology 245:244-245.

(79) Shane, B., Watson, J. E., and Stokstad, E. L. R.: Uptake and metabolism of ^{3}H-folate by normal and by vitamin B-12 and methionine-deficient rats. Biochem. Piophys. Acta. 497:241-252, 1977.

(80) Gaul, G. E.,ed., Biology of Brain Dysfunction. New York. Plenum Press, 1973.

(81) Stryer, L.: Biochemistry. 2nd ed. Freeman. Toppan Co., 1981.

(82) Chida, N., Hirono, H., and Arakawa, T.: Effects of dietary folate deficiency on fatty acid composition of myelin cerebroside in growing rate. Tohoku, J. Exp. Med. 108:219-224, 1972.

(83) Akesson, B., Fehling, M., Jagerstad, M., and Stenram, U.: Effect of experimental folate deficiency on lipid metabolism in liver and brain. Brit. J. Nutr. 47:505-520, 1982.

(84) Baker, S. J.: Nutriton and diseases of the blood: the megaloblastic anemias. Prog. Food Nutr. Sci. 1:421-459, 1975.

(85) Noronha, J. M., and Silverman, M.: Vitamin B_{12} and intrinsic factor. 2nd European Symposium. Editor: Heinrich, H. C., p. 728, F. Enke. Verlag, Switzerland, 1962.

(86) Herbert, V., and Zalusky, R.: Interrelationsof vitamin B_{12} and folic acid metabolism. Folic acid clearance studies. J. Clin. Invest. 41:1263-1276, 1962.

(87) Cooper, B. A., and Lowenstein, L.: Relatively folate deficiency of erythrocytes in pernicious anemia and its correction with cyanocobalamin. Blood 24:502-521, 1964.

(88) Smith, R. M., Osborne-White, W. S., and Gawthrone, J. M.: Folic acid metabolism in vitamin B_{12}-deficient sheep. Effect of injected methionine on liver sonstituents. Associated with folate metabolism. Biochem. J. 142:105-117, 1974.

(89) Gawthorne, J. M., and Stokstad, E. L. R.: The effect of vitamin B_{12} and methionine on folic acid uptake by rat liver. Proc. Soc. Exp. Biol. Med. 136:42-46, 1971.

(90) O'Broin, J. D., Temperley, I. J., and Scott, J. M.: Erythrocyte, plasma, and serum folate: specimen stability before microbiological assay. Clin. Chem. 26:522-524, 1980.

(91) Asquith, P., Oilbaum, H. M., and Dawson, D. W.: Scorbutic magaloblastic anemia responding to ascorbic acid alone. Brit. Med. J. 4:402, 1967.

(92) Brown, A.: Megaloblastic anemia associated with adult scurvy. Report of a case which responded to synthetic ascorbic acid alone. Brit. J. Haematol. 1:345-351, 1955.

(93) Zalusky, R., and Herbert, V.: Megaloblastic anemia in scurvy with response to 50 microgram of folic acid daily. New Engl. J. Med. 265:1033-1038, 1961.

(94) Stokes, P. L., Melikian, V., Leeming, R. L., Portmangraham, H., Blair, J. A., and Cooke, W. J.: Folate metabolism in scurvy. Am. J. Clin. Nutr. 28:126-129, 1975.

(95) Hershko, C., Crossowicz, N., Rachmilewitz, M., Kesten, S., and Izak, G.: Serum and erythrocyte folate in combined iron and folate deficiency. Am. J. Clin. Nutr. 28:1217-1222, 1975.

(96) Roberts, P. D., St. John, D. J. B., Sinha, R., Stewart, J. S., Baird, I. M., Coghill, N. F., and Morgan, J. O.: Apparent folate deficiency in iron-deficieny anemia. Brit. J. Haematol. 20:165-176, 1971.

(97) Baker, S. J.: Nutritional anemia - a major controllable public health problem. Bull. WHO. 56:659-675, 1978.

(98) Elsborg, L., Rosenquist, A., and Helms, P.: Iron intake by teenage girls and by pregnant women. Intern. J. Vit. Nutr. Res. 49:210-214, 1979.

(99) Elsborg, L., and Rosenquist, A.: Folate intake by teenage girls and by pregnant women. Intern. J. Vit. Nutr. Res. 49:70-76, 1979.

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

(101) Toskes, P. P., Smith, G. W., Bensinger, T. A., Giannella, R. A., and Conrad, M. E.: Folic acid abnormalities in iron deficiency: The mechanism of decreased serum folate levels in rats. Am. J. Clin. Nutr. 27:355-361, 1974.

(102) Celada, A., Rudolf, H., and Donath, A.: Effect of experimental chronic alcohol ingestion and folic acid deficiency in iron absorption. Blood 54:906-915, 1979.

(103) Alperin, J. B., Hutchinson, H. T., and Levin, W. C.: Studies of folic acid requirements in megaloblastic anemia of pregnancy. Arch. Inter. Med. 117:681-688, 1966.

(104) Scott, J. M.: Iron-sorbital-citrate in pregnancy anemia. Brit. Med. J. 2:354-357, 1963.

(105) Tamura, T., Shane, B., Baer, M. T., King, J. C., Margen, S., and Stokstad, E. L. R.: Absorption of mono- and polyglutamyl folates in zinc-depleted man. Am. J. Clin. Nutr. 31:1984-1987, 1978.

(106) Silink, M., Reddil, R., Bethell, M., and Rowe, P. B.: γ-Glutamyl hydrolase (conjugase) purification and properties of the bovine hepatic enzyme. J. Biol. Chem. 250:5982-5993, 1975.

(107) Bovina, C., Landi, L., Pasquali, P., and Marchetti, M.: Biosynthesis of folate coenzymes in riboflavin-deficient rats. J. Nutr. 99:320-324, 1969.

(108) Dako, D. Y., and Hill, D. C.: Effect of riboflavin and folic acid supplementation on some haematopoietic parameters in the rat. Intern. J. Vit. Nutr. Res. 50:254-260, 1980.

(109) Vitale, J. J., and Hegsted, D. M.: Effects of dietary methionine and vitamin B12 deficiency on folate metabolism. Brit. J. Haematol. 17:467-475, 1969.

(110) Buehring, K. U., Butra, K. K., and Stokstad, E. L. R.: The effect of methionine on folic acid and histidine metabolism in purfused rat liver. Biochem. Biochys. Acta. 279:498-512, 1972.

(111) Bonjour, J. P.: Vitamins and alcoholism. II. Folate and vitamin B12. Intern. J. Vit. Nutr. Res. 50:96-121, 1980.

(112) Wu, A., Chanarin, I., and Levi, A. J.: Macrocytosis of chronic alcoholism. Lacet 1:829-830, 1974.

(113) Sullivan, L. W., and Herbert, V.: Suppression of hematopoiesis by ethanol. J. Clin. Invest. 43:2048-2062, 1964.

(114) Paine, C. J., Eithner, E. R., and Dickson, V.: Concordance of

radioassay and microbiological assay in the study of the ethano-induced fall in serum folate level. Am. J. Med. Sci. 266: 135-138, 1973.

(115) Li, T. K., Schenker, S., and Lumeng, L.: Alcohol and Nutrition. Research monograph No. 2, Rockville, MD. NIAAA, 1979.

(116) Herbert, V.: The five possible causes of all nutrient deficiency: illustrated by deficiencies of vitamin B_{12} and folic acid. Am. J. Clin. Nutr. 26:77-86, 1973.

(117) Herbert, V.: Experimental nutritional folate deficiency in man. Trans. Asso. Am. Physicians Phila. 75:307-320, 1962.

(118) Reynolds, E. H.: Neurological aspects of folate and vitamin B_{12} metabolism. Clin. Haematol. 5:661-696, 1976.

(119) Chanarin, I., Mollin, D. L., and Anderson, B. B.: The clearance from plasma of folic acid injected intravenously in normal subjects and patients with megaloblastic anemia. Brit. J. Haematol. 4:435-446, 1958.

(120) Zittoun, J., Marquet, J., Zittoun, R.: Effect of folate and cobalamin compounds on the deoxyuridine suppression test in vitamin B_{12} and folate deficiency. Blood 51:119-127, 1978.

(121) Water, S. H., and Mollin, D. L.: Studies on the folic acid activity of human serum. J. Clin. Path. 14:335-344, 1961.

(122) Banerjee, D. K., and Chatterjea, J. B.: Observations on the lability of serum, whole blood and RBC folates of human blood. Indian J. Med. Res. 59:369-376, 1971.

(123) Bird, O. D., Mcglohon, V. N., and Vaitkus, J. W.: Naturally occuring folates in the blood and liver of rats. Anal. Biochem. 12:18-35, 1965.

(124) Hofflbrand, A. V., Newcombe, B. F. A., and Mollin, D. L.: Method of assay of red cell folate activity and the value of the assay as a test for folate deficiency. J. Clin. Path. 19:17-28, 1966.

(125) Chanarin, I., Bennett, M., and Berry, V.: Urinary of histidine derivatives in megaloblastic anemia and other conditions and a comparison with the folic acid clearance test. J. Clin. Path. 15:269-273, 1962.

(126) Vitale, J. J., Streiff, R. R., and Hellerstein, E. E.: Folate metabolism and iron deficiency. Lacet 2:393-394, 1965.

(127) Mohamed, S. D., and Roberts, M.: Abnormal histidine metabolism in thyrotoxicosis in man. A possible index of impaired folacin function. Lancet 2:933-935, 1965.

(128) Waxman, S., Schrecker, C., and Herbert, V.: Radioisotopic assay

for measurement of serum folate levels. Blood 38:219-228, 1971.

(129) Usdin, E.: Blood folic acid studies. VI. Chromatographic resolution of folic acid active substances obtained from blood. J. Biol. Chem. 234:2373-2376, 1954.

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

(131) Herbert, V.: Minimal daily adult folate requirement. Arch. Intern. Med. 110:649-652, 1962.

(132) Miller, A. L.: Microbiological Laboratory Techniques. Lexingto , Massachusetts, D.C. heath and Company, 1976.

(133) Flynn, L. M., Williams, V. B., O'Dell, B. L., and Hogan, A.: Medium for assay of vitamins with lactic acid bacterial. Anal. Chem. 23:180-185, 1951.

(134) SAS Institute: SAS User's Guide, 1979 ed. Box 8000, Cary, North Carolina. SAS Institute Inc., 1979.

(135) Toepfer, E. W., Zook, E. G., Orr, M. L., and Richardson, L. R.: Folic acid content of foods. USDA Agriculture Handbook No. 29, Washington, D.C., 1952.

(136) Dawson, D. W., and Geary, C.: Hepatic and serum folates in patients fasting and after oral folic acid. J. Clin. Path. 24: 129-130, 1971.

(137) Theriault, L., and Page, M.: A solid-phase enzyme immuoassay for serum ferritin. Clin. Chem. 23:2142-2144, 1977.

(138) Johnson, L. D., Bachman, J. G., and O'Malley, P. M.: Drugs and the Class of '78: Behaviors, Attitudes, and Recent National Trends. National Institute on Drug Abuse. DHEW Pub. No.(ADM)79-877, 1979.

(139) Tanner, J. M.: Growth at Adolescence. 2 ed. Oxford. Blackwell Scientific Publication Ltd., 1962.

(140) Streiff, R. R.: Folate deficiency and oral contraceptives. Am. J. Med. A. 214:105-108, 1970.

APPENDIXES

APPENDIX A

QUESTIONNAIRES

Form D 1 b

Subject no._____ Subject name_____

RECORD SHEET FOR DIETARY RECALL. "Tell me, beginning with the time you got up yesterday morning, everything you ate or drank until you went to bed last night."

(Bracket foods eaten together. Note carefully times of first & last meals or snacks.)

FOOD AND DESCRIPTION	AMOUNT	FREQUENCY	TIME OF DAY	PLACE

WHAT SUPPLEMENTS, IF ANY, DID YOU TAKE YESTERDAY? HOW MANY? WHAT TIME?
(Get brand name. Ask if multivitamins contain iron.)

SUPPLEMENT	AMOUNT/TABLET	NO. TABLETS	TIME OF DAY

GO TO NO. 39, page 9.

S-150 REGIONAL PROJECT

FORM A3-A

SUBJECT NO. _____

DATE _____

ANTHROPOMETRIC MEASUREMENTS

MEASUREMENTS TAKE BY: 1 NUTRITIONIST 2 ANTHROPOLOGIST 3 NURSE
4 STUDENT 5 OTHER (SPECIFY) _____

NOTE: ASK SUBJECT TO REMOVE SHOES AND ALL HEAVY OUTER GARMENTS.

1. BIRTHDATE _____/_____/_____. CALCULATE AGE TO THE NEAREST MONTH. RECORD.
MONTH DAY YEAR

2. WEIGHT _____LBS / 2.2 = _____ KG
RECORD WEIGHT IN KILOGRAMS

3. CLOTHING ESTIMATE _____ G
(NOTE CLOTHING LIST IN #7)

4. HEIGHT _____ CM

5. TRICEPS CIRCUMFERENCE _____ CM

6. TRICEPS SKINFOLD _____ MM

7. CHECK THE CLOTHING ITEMS WORN WHEN SUBJECT WAS WEIGHED IN ORDER TO OBTAIN CLOTHING ESTIMATE. CALCULATE AND RECORD IN #3.

CLOTHING LIST:

BRA: NATURAL (25 G) PADDED (40 G)

PANTIES: NYLON (15 G) COTTON (20 G)

SLIP: FULL (110 G) HALF (80 G)

SOCKS: FOOTLETS (30 G) SHORT SOCKS (35 G) KNEE SOCKS (50 G)

SHEER HOSE: KNEE LENGTH (25 G) PANTY HOSE (60 G)

SLACKS: POLYESTER (250 G) COTTON (360 G) JEANS (440 G)

SKIRT: LIGHT (250 G) MEDIUM (350 G) HEAVY (420 G)

BLOUSE: LIGHT (100 G) MEDIUM (190 G) HEAVY (280 G)

SWEATER: LIGHT (320 G) MEDIUM (390 G) HEAVY (440 G)

BELT: LIGHT (60 G) MEDIUM (100 G) HEAVY (140 G)

OTHER: LIST AND WEIGH SIMILIAR ITEMS

_____

_____

S-150 REGIONAL PROJECT

FORM S 1

SUBJECT _____ ____

DATE _____

SOCIO DEMOGRAPHIC BACKROUND INFORMATION

RESPONDENT'S RELATION TO SUBJECT_____

***NOTE TO INTERVIEWER: IF SUBJECT DOES NOT LIVE WITH PARENT(S) AND/OR GUARDIAN(S) SHE MAY POSSIBLY BE THE RESPONDENT. CHANGE WORDING OF ITEMS APPROPRIATELY.

1. SUBJECT'S RACE
   1=WHITE 2=BLACK

2. LOCATION OF SUBJECT'S RESIDENCE
   1=MAJOR URBAN- GREATER THAN OR EQUAL TO 100,000
   2=MINOR URBAN- GREATER THAN OR EQUAL TO 2500 AND LESS THAN 100,000
   3=RURAL,NON-FARM- LESS THAN 2500 AND NON-FARMING
   4=RURAL,FARM- LESS THAN 2500 AND FARMING

   CODE THE LOCATION OF SUBJECT'S RESIDENCE TO THE RIGHT

3A. DOES SUBJECT ATTEND SCHOOL?
   1=YES 2=NO (IF NO, GO TO QUESTION 4)

3B. WHAT IS SUBJECT'S GRADE IN SCHOOL?
   (RECORD ACTUAL GRADE-EXAMPLE GRADE 8=08)

4. IF SUBJECT IS NOT ENROLLED IN SCHOOL. ASK:

   WHAT WAS THE LAST GRADE COMPLETED?
   (RECORD ACTUAL GRADE-EXAMPLE GRADE 8=08)

5A. IS SUBJECT EMPLOYED?
   1=YES 2=NO

5B. IF EMPLOYED, IS SUBJECT EMPLOYED
   1=FULL-TIME 2=PART-TIME

5C. IF EMPLOYED, WHAT IS SUBJECT'S OCCUPATION?_____

***NOTE: REFER TO TABLE VII-OCCUPATIONS: LEVELS AND KINDS- IN CODING THE REPLY TO THIS QUESTION.

1=PROFESSIONAL	5=BLUE COLLAR
2=PROPRIETOR	6=SERVICE
3=BUSINESS	7=FARM
4=WHITE COLLAR	8=OTHER

6. IS SUBJECT
   1=MARRIED 2=WIDOWED 3=DIVORCED 4=SEPARATED
   5=NEVER MARRIED

S-150 REGIONAL PROJECT

FORM S 1 , CONT.

SUBJECT NO. ___ ___ STATE ____ STATION _____

16A. NOW THAT YOU HAVE MENTIONED THE SOURCE(S) OF YOUR FAMILY INCOME, WHAT IS THE TOTAL (ADD ALL SOURCES) BEFORE TAXES ARE DEDUCTED? YOU CAN DO THIS BY WEEK, MONTH OR YEAR.

$_____WEEKLY

$_____MONTHLY

$_____YEARLY

***NOTE: IF LISTED WEEKLY OR MONTHLY ASK 16B.

16B. HOW MANY WEEKS OR MONTHS OF THE YEAR DO YOU MAKE THIS AMOUNT?

_____WEEKS

_____MONTHS

17. GIVEN THE ABOVE INFORMATION IN QUESTION 16 WHAT IS THE SUBJECTS TOTAL GROSS FAMILY INCOME?
(RECORD ACTUAL AMOUNT)

18. HOW MANY PEOPLE DOES THIS INCOME SUPPORT?
(RECORD ACTUAL NUMBER)

S-150 REGIONAL PROJECT

FORM A2

SUBJECT NO. _____. ____

DATE _____

MEDICAL HISTORY
(TO BE ASKED OF SUBJECT)

WE WOULD LIKE TO ASK YOU THE FOLLOWING QUESTIONS BECAUSE THE MENSTRUAL CYCLE AND CERTAIN DRUGS CAN AFFECT THE OUTCOME OF SOME OF THE ANALYSES WE ARE DOING. YOUR REPLIES WILL BE KEPT VERY CONFIDENTIAL.

1. HAVE YOUR STARTED YOUR MENSTRUAL PERIODS? 1 = YES 2 = NO
   IF YES, ANSWER QUESTIONS 2-5B. (IF NO, OMIT.)

2. HOW OLD WERE YOUR WHEN YOUR MENSTURAL PERIODS STARTED?
   AGE IN YEARS AND MONTHS _____
   (INTERVIEWER CALCULATE AND RECORD IN MONTHS.)

3. ARE YOUR MENSTRUAL PERIODS REGULAR? 1 = YES 2 = NO
   IF YES, WHAT IS THE LENGTH OF TIME BETWEEN THE FIRST DAY OF ONE PERIOD AND THE FIRST DAY OF THE NEXT ONE? _____
   _____ NUMBER OF DAYS.
   (INTERVIEWER CALCULATE AND RECORD. IF IRREGULAR, LEAVE BLANK.)

4. DATE WHEN LAST PERIOD STARTED _____/_____/_____
   DAY MONTH YEAR
   _____ DAY IN CYCLE.
   (INTERVIEWER CALCULATE AND RECORD.)

5. DO YOU TAKE MEDICATION FOR ANY OF THE FOLLOWING?
   IF YES TO A, B, OR C, PLEASE SPECIFY THE NAME OF THE MEDICATION.

   1 = YES 2 = NO

   A. PAIN RELATED TO MENSTRUATION SPECIFY _____

   B. TO CONTROL REGULARITY OR FLOW OF MENSTRUATION
   SPECIFY _____

   C. TO CONTROL ACNE SPECIFY _____

6. HAVE YOU TAKEN BIRTH CONTROL PILLS? 1 = YES 2 = NO
   IF YES, ANSWER PARTS A-C.

   A. ARE YOU TAKING BIRTH CONTROL PILLS NOW? 1 = YES 2 = NO

   B. IF YOU ARE NOT TAKING THEM NOW, HOW LONG SINCE YOU STOPPED TAKING THEM?

   _____ NUMBER OF MONTHS (RECORD)

   C. IF YOU HAVE TAKEN THE PILL, WHAT IS THE TOTAL LENGTH OF TIME YOU TOOK IT?

   _____ NUMBER OF MONTHS (RECORD)

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FORM A2, CONT.

SUBJECT NO. _____ __ STATE _____ STATION _____

7. IF YOU ARE PRESENTLY TAKING BIRTH CONTROL PILLS, WHAT TYPE DO YOU TAKE?

00. DO NOT KNOW
01. BREVICON, MIDICON, OVCON-35
02. ENOVID E OR #21
03. DEMULEN
04. LOESTRIN 1.5/30
05. LOESTRIN 1/20
06. NORINYL 1/50, NORLESTRIN 1/50, OVCON-50, ORTHO-NOVUM 1/50
07. NORINYL 1/80, ORTHO-NOVUM 1/80
08. OVRAL
09. OVULEN 21 OR OVULEN 28
10. OTHER, SPECIFY _____

8. HOW OFTEN DO YOU USE EACH OF THE FOLLOWING? WE NEED TO KNOW SINCE THEY COULD AFFECT THE WAY THE BODY USES FOOD.

A. SMOKING TOBACCO
(RECORD # OF TIMES EACH DAY. IF NEVER, RECORD 00.)

WHICH BEST DESCRIBES YOUR PATTERN FOR:

0 = NEVER 1 = RARELY 2 = 1X/MO 3 = 2X/MO
4 = 1-2X/WK 5 = 3-6X/WK 6 = 1X/DAY 7 = 2X OR +/DAY

B. USING POT (MARIJUANA) _____

C. DRINKING WINE (SERVING = 4 OZ.) _____

D. DRINKING BEER (SERVING = 7 OZ.) _____

E. DRINKING HARD LIQUOR (SERVING = 1 OZ.) _____

F. OTHER DRUGS, SPECIFY _____ _____

9. FOR EACH OF THE ABOVE USED, HOW LONG HAVE YOU USED THEM. (RECORD TIME AS MONTHS TO NEAREST MONTH STARTING WITH 1).

A. SMOKING TOBACCO _____

B. POT _____

C. WINE _____

D. BEER _____

E. HARD LIQUOR _____

D. OTHER DRUGS _____

10. HAVE YOU TAKEN AN ANTIBIOTIC DURING THE PAST WEEK?
1 = YES 2 = NO

S-150 REGIONAL PROJECT

FORM D1, CONT.

SUBJECT NO. _____ _____ STATE _____ STATION _____

47. HOW MANY TIMES EACH YEAR DO YOU GO ON A WEIGHT REDUCTION DIET?

48. HOW LONG DOES THE DIET USUALLY LAST (SELECT ONE).

1. LESS THAN ONE MONTH
2. ONE TO THREE MONTHS
3. FOUR TO SIX MONTHS
4. MORE THAN SIX MONTHS

49. HAVE YOU EVER BEEN ON A DIET TO TRY TO GAIN WEIGHT?
1 = YES 2 = NO

IF ANSWER TO 'NO', SKIP TO #52.

50. HAVE YOU TRIED TO GAIN WEIGHT WITHIN THE PAST YEAR?
1 = YES 2 = NO

51. IF YES, WAS IT RECOMMENDED OR DECIDED ON PRIMARILY BY (SELECT ONE).

1. PHYSICIAN
2. MOTHER
3. FATHER
4. SELF
5. GIRLFRIEND(S)
6. BOYFRIEND(S)
7. MEDIA

52. ARE YOU PRESENTLY TRYING TO _____ WEIGHT?
GAIN = 1 LOSE = 2 NEITHER = 3

53. DO YOU THINK YOUR WEIGHT IS NOW (CIRCLE ONE):
TOO HEAVY = 3 TOO LIGHT = 1 ABOUT RIGHT = 2

54. DO YOU ADD SALT TO YOUR FOOD AT THE TABLE (CIRCLE ONE):
ALMOST ALWAYS AND BEFORE TASTING = 4 SOMETIMES = 3
ALMOST ALWAYS BUT ONLY AFTER TASTING = 2 ALMOST NEVER = 1

55. DO YOU LIKE VERY SALTY FOODS SUCH AS SALTED NUTS, POTATO CHIPS?
YES = 1 NO = 2

56. WHO PREPARES BREAKFAST IF YOUR FAMILY USUALLY (AT LEAST 4 TIMES EACH WEEK) HAS A PREPARED MEAL? (SELECT ONE.)

0. MEAL IS NOT PREPARED AT LEAST 4 TIMES EACH WEEK
1. MOTHER
2. FATHER
3. GRANDMOTHER, AUNT, OR OTHER FEMALE RELATIVE
4. YOURSELF
5. OTHER CHILDREN IN THE FAMILY
6. MOTHER PREPARES FOR THE FAMILY AND THE FATHER PREPARES HIS OWN
7. EACH PERSON PREPARES HIS/HER OWN
8. VARIES FROM DAY TO DAY
9. OTHER; SPECIFY _____

APPENDIX B

RAW DATA FOR VARIABLES

ABBREVIATIONS FOR VARIABLE NAMES

SUBJ = subject number.
SFOL = serum folacin concentration (ng/ml).
FOL = dietary folacin (μg).
RES = place of residence: 1 = city, 2 = town, 3 = rural area.
HT = height (cm).
MENS = menstrual status: 1 = post-menarcheal, 2 = pre-menarcheal.
PCINC = per capita income ($ per year)
TOB = use of tobacco: 0 = never, 1 = rarely, 2 = 1x/mo, 3 =2x/mo, 4 = 1-2x/wk, 5 = 3-6/wk, 6 = 1x/day, 7 = 2x or more/day.
ALC = use of alcohol: 0 = never, 1 = rarely, 2 = 1x/mo, 3 =2x/mo, 4 = 1-2x/wk, 5 = 3-6/wk, 6 = 1x/day, 7 = 2x or more/day.
HB = hemoglobin concentration (g/dl).
VB = dietary vitamin B_{12} (μg).
VC = dietary vitamin C (mg).
FE = dietary iron (mg).
ZN = dietary zinc (mg).
RB = dietary riboflavin (mg).
MET = dietary methionine (g).
SUC = sucrose (g).
RACE: 1 = white, 2 = black.
AGE = age (month).
ENERGY = dietary energy (kcal).
DIET = dieting practice: 1 = trying to gain weight, 2 = trying to lose weight, 3 = neither.
SUPP = nutritional supplements: 1 = use, 2 = non-use.
WT = nude weight (kg).
FOLRDA = % RDA for folacin intake.

OBS	SUBJ	SFOL	FOL	RES	HT	MENS	PCINC	TOB	ALC	HB	VB	VC	FE	ZN	RB	MET	SUC	RACE	AGE	ENERGY	SUPP	WT	DIET	FOLRDA
1	1	6.4	170	3	153.0	2	2200	0	1	13.01	3.03	166	14.9	13.7	1.54	1.72	37	1	146	2028	0	38.5	3	42.50
2	2	10.2	386	2	169.5	1	6580	5	4	13.51	5.08	78	20.2	9.3	2.30	1.15	43	1	189	2178	1	54.1	2	96.50
3	3	10.6	203	2	158.8	2	1833	1	2	13.72	3.83	66	13.8	13.5	1.98	1.75	74	1	168	2281	0	56.7	2	50.75
4	4	23.8	454	2	168.3	1	9333	0	1	14.77	2.47	275	13.8	8.0	1.83	1.17	105	1	187	2605	0	56.6	3	113.50
5	5	2.8	230	2	154.9	1	8500	0	1	13.92	2.33	44	7.9	6.8	1.10	3.06	41	1	162	1170	0	54.5	3	57.50
6	6	50.0	407	2	157.5	1	4080	0	2	13.56	8.66	81	27.2	10.1	3.34	1.16	37	1	189	1622	1	46.9	2	101.75
7	7	9.0	268	2	151.8	2	846	0	1	13.20	4.75	146	15.9	19.4	2.20	3.54	78	1	166	2515	0	53.8	2	67.00
8	8	15.5	136	2	161.9	1	4167	0	0	13.08	1.19	93	5.5	6.1	0.66	0.69	.	1	193	1078	0	57.1	2	34.00
9	9	16.6	88	2	153.7	2	3800	0	1	13.93	3.37	29	10.3	10.3	1.62	2.66	69	1	174	1673	0	43.1	3	22.00
10	10	2.6	94	3	161.3	1	20000	0	1	14.72	1.25	45	5.2	5.0	0.59	0.68	70	1	163	1195	0	67.2	2	23.50
11	11	0.8	128	3	157.5	1	10000	0	1	13.31	1.48	26	6.0	5.3	0.75	0.60	30	1	189	1109	0	88.4	2	32.00
12	12	14.5	43	2	155.6	1	2500	0	4	14.39	1.52	27	9.2	10.5	0.81	3.57	3	1	193	1271	0	54.3	3	10.75
13	13	10.5	182	2	160.0	1	6400	0	0	13.46	2.65	116	8.8	7.6	1.42	0.94	41	1	172	1572	0	57.8	3	45.50
14	14	0.6	362	2	158.8	1	10000	0	0	13.97	7.60	184	27.6	15.6	3.56	1.87	84	1	165	2368	0	41.2	1	90.50
15	15	19.2	138	3	169.5	1	10000	0	0	13.61	4.61	56	13.1	14.3	2.01	2.01	53	1	164	2124	0	56.0	3	34.50
16	16	5.6	376	2	155.6	1	10354	0	2	14.06	4.05	534	9.0	6.8	1.70	1.50	26	1	162	1132	0	58.4	3	94.00
17	17	38.3	118	2	165.1	1	1580	0	1	13.11	3.27	27	15.7	18.1	1.65	2.25	72	1	175	2188	0	53.6	3	29.50
18	18	28.8	84	3	158.4	1	8000	0	1	13.27	0.97	74	6.6	5.9	0.62	2.33	45	1	162	1005	0	46.8	3	21.00
19	19	9.0	311	2	148.0	2	3500	0	0	14.01	3.35	82	13.1	8.2	1.76	1.20	36	1	149	1628	0	38.2	3	77.75
20	20	14.9	400	2	143.5	2	3240	0	1	13.10	12.89	230	24.7	14.7	3.51	2.31	107	1	165	2915	1	45.0	2	100.00
21	21	13.7	183	2	162.6	1	4167	0	0	14.21	1.68	125	6.0	6.9	0.96	1.95	55	1	193	1291	0	54.9	2	45.75
22	22	50.0	304	2	158.8	1	7200	0	3	14.34	5.85	77	22.6	15.6	2.35	2.84	53	1	193	2035	1	47.8	3	76.00
23	23	11.6	30	2	158.1	1	5200	0	4	14.62	1.60	17	2.7	4.1	0.79	0.58	49	1	179	945	0	51.6	.	7.50
24	24	15.8	24	3	159.4	1	5750	0	3	13.61	1.19	15	5.5	5.2	0.73	0.77	58	1	174	1198	0	51.0	2	6.00
25	25	6.2	168	2	163.8	1	5040	0	0	15.00	0.84	128	6.3	5.3	0.61	0.74	34	1	173	1085	0	54.0	3	42.00
26	26	7.4	357	2	159.4	1	6000	0	4	12.07	10.17	175	25.6	8.7	3.65	1.49	90	1	198	2488	1	44.1	3	89.25
27	27	19.4	159	2	162.6	1	8800	7	2	15.05	2.10	18	4.9	5.6	1.10	0.83	15	1	173	819	0	70.1	3	39.75
28	28	4.1	170	2	167.6	1	3960	3	1	14.76	2.08	83	12.4	24.8	1.14	1.39	137	1	195	2512	0	70.0	3	42.50
29	29	2.8	230	3	165.1	1	8667	0	0	14.72	4.74	37	17.5	7.7	1.51	0.81	68	1	164	1138	1	58.7	3	57.50
30	30	1.3	194	2	163.8	1	1167	0	0	14.12	2.50	38	7.9	9.5	1.17	1.17	42	1	173	1483	0	55.9	3	48.50
31	31	3.0	226	3	156.2	2	6000	0	1	13.06	4.61	37	12.9	18.7	2.14	2.24	37	1	143	2020	0	39.8	3	56.50
32	32	4.7	389	2	170.2	1	11250	0	1	11.84	5.96	58	11.0	10.5	2.33	2.74	41	1	188	2067	0	53.2	3	97.25
33	33	8.7	237	2	155.6	2	3725	0	1	12.53	4.71	89	11.8	16.5	2.04	2.27	54	1	148	2052	0	41.7	3	59.25
34	34	7.2	169	2	160.7	1	8571	0	2	14.23	2.11	35	11.2	15.4	1.46	1.31	125	1	188	2458	0	50.2	3	42.25
35	35	3.5	108	3	157.5	1	6052	0	0	12.69	3.20	39	10.8	9.1	1.78	1.07	31	1	172	1673	0	51.9	3	27.00
36	36	19.0	131	3	148.6	2	5000	0	0	13.94	6.14	42	20.9	13.4	2.79	1.59	112	1	144	2556	0	41.4	2	32.75
37	37	1.8	73	3	162.6	1	2857	4	2	15.45	4.32	6	7.8	7.0	1.88	1.41	16	1	189	1976	0	59.4	2	18.25
38	38	36.4	189	3	153.7	2	1349	0	1	13.62	3.36	39	12.3	11.5	1.94	1.57	59	2	146	2373	0	45.4	2	47.25
39	39	9.6	174	3	161.3	1	1349	0	0	11.94	3.49	16	18.0	15.0	1.87	1.94	65	2	168	2538	0	51.7	2	43.50
40	40	58.5	310	3	148.6	2	5100	0	1	12.54	3.11	72	7.5	7.0	1.60	1.26	38	1	140	1382	0	48.2	2	77.50
41	41	47.7	80	3	164.5	1	4398	0	1	13.52	16.65	643	9.7	33.4	17.10	1.50	22	1	147	1472	0	68.8	3	20.00
42	42	1.5	142	3	165.1	1	4667	0	0	14.10	3.01	86	11.5	11.0	1.13	1.68	14	1	169	1657	0	85.9	2	35.50
43	43	3.5	107	3	151.1	2	8000	0	1	12.75	6.88	20	14.1	16.3	2.63	3.64	55	1	140	2479	0	39.9	3	26.75
44	44	68.4	831	2	148.6	2	2667	0	1	14.83	7.11	49	14.6	12.2	3.67	2.17	99	1	150	2727	0	36.7	3	207.75
45	45	2.1	65	2	165.7	1	7000	0	1	13.80	4.04	49	10.7	9.6	0.91	1.06	97	1	188	1723	0	56.4	2	16.25
46	46	3.2	63	3	164.5	1	8600	0	1	12.62	2.77	76	9.9	6.5	1.36	1.44	69	1	171	1643	0	52.6	2	15.75
47	47	0.8	115	3	174.0	1	8600	0	0	13.78	3.95	24	10.5	13.1	1.76	1.49	55	1	191	1739	0	60.1	3	28.75
48	48	18.2	47	2	162.6	1	5917	0	4	13.51	0.58	21	6.7	3.8	0.51	0.63	53	1	190	1418	0	55.1	2	11.75
49	49	3.5	54	3	161.3	1	5000	0	1	13.56	1.49	59	3.8	3.3	0.61	0.49	61	1	196	822	0	47.3	2	13.50
50	50	11.8	126	3	156.2	1	5800	0	1	13.88	1.23	84	4.4	3.5	0.67	0.57	42	1	174	948	0	63.8	2	31.50
51	51	36.4	387	3	158.8	2	9667	0	0	14.18	7.52	99	26.4	18.4	3.04	1.97	64	1	150	2058	1	45.8	3	96.75
52	52	7.4	186	3	144.1	2	6667	1	2	14.86	6.78	68	20.5	34.7	2.64	13.60	175	1	148	4478	0	37.0	2	46.50
53	53	2.6	89	2	172.1	1	6250	0	0	11.65	3.83	36	10.5	9.5	1.65	1.33	93	1	190	1551	0	62.5	3	22.25
54	54	5.2	80	3	162.6	1	5000	0	3	13.85	2.25	45	6.0	4.5	0.82	0.65	26	1	186	1081	0	44.6	3	20.00
55	55	32.4	389	2	156.8	2	8000	0	0	14.25	10.26	117	7.8	9.6	3.41	1.83	38	1	150	1690	0	55.7	3	97.25

OBS	SUBJ	SFOL	FOL	RES	HT	MENS	PCINC	TOB	ALC	HB	VB	VC	FE	ZN	RB	MET	SUC	RACE	AGE	ENERGY	SUPP	WT	DIET	FOLRDA
56	56	0.8	595	2	169.5	1	8000	0	2	12.87	8.49	128	10.9	11.4	2.95	1.55	55	1	187	1747	1	62.0	2	148.75
57	57	10.7	179	3	141.6	2	6000	0	1	13.79	2.52	132	8.3	8.8	1.34	1.30	55	1	149	1419	0	32.8	2	44.75
58	58	7.2	274	2	148.0	2	7500	0	1	14.35	7.15	76	23.7	12.4	2.51	1.34	111	1	141	2123	0	39.4	3	68.50
59	59	1.2	92	2	158.1	2	6250	0	0	15.21	2.37	20	7.5	8.8	1.26	1.11	81	1	144	1280	0	59.6	2	23.00
60	60	4.2	545	2	156.2	1	7000	0	0	12.44	7.19	66	18.4	14.3	2.85	1.85	77	1	168	2298	0	45.0	3	136.25
61	63	18.2	128	2	151.1	2	7000	0	1	13.75	1.91	306	6.6	6.8	0.90	0.92	30	1	141	1261	0	47.1	3	32.00
62	64	8.6	94	2	156.8	1	7000	0	0	13.61	2.26	15	9.4	6.9	1.08	0.85	91	1	168	1647	0	45.4	2	23.50
63	65	3.8	308	2	152.4	2	5000	0	0	13.44	5.90	98	11.8	11.1	2.63	1.93	139	1	142	3040	0	34.6	3	77.00
64	66	8.7	173	2	156.2	1	2229	0	0	14.62	2.38	82	8.3	6.0	1.21	0.90	70	1	150	1429	0	55.2	2	43.25
65	67	0.9	146	2	169.5	1	9000	0	2	13.75	1.05	27	8.5	8.0	1.04	0.84	80	1	173	1634	0	62.6	2	36.50
66	68	1.3	411	2	166.4	1	5520	0	0	14.48	4.02	28	15.8	9.3	2.21	1.08	50	1	174	1749	0	54.1	3	102.75
67	69	6.4	130	3	156.8	1	4600	0	0	13.42	3.63	23	6.1	7.2	1.88	1.07	65	1	148	1293	0	46.0	2	32.50
68	70	.	34	2	.	.	17500	.	.	.	2.32	29	8.3	8.0	0.59	0.97	37	1	149	1306	0	.	.	8.50
69	71	14.4	419	3	153.0	2	17500	0	1	16.31	1.73	165	12.1	12.5	1.00	1.52	170	1	143	2812	0	45.3	3	104.75
70	72	20.5	68	2	160.0	1	12500	0	0	13.22	0.95	47	2.7	1.6	0.35	0.34	8	1	198	483	0	51.7	3	17.00
71	73	.	111	2	.	.	1047	.	.	.	2.88	128	7.6	7.0	1.46	1.27	98	2	142	1497	0	.	.	27.75
72	74	18.3	318	2	158.8	1	4800	0	0	13.66	8.23	101	16.3	7.7	2.76	1.07	33	2	141	1904	1	46.0	3	79.50
73	75	5.8	217	3	160.0	1	3200	1	1	13.35	2.22	111	8.8	6.1	1.34	1.14	68	1	197	1480	0	58.0	2	54.25
74	76	5.2	296	2	154.3	2	5520	0	0	14.88	4.46	61	14.5	8.7	2.55	1.38	129	1	147	2432	0	38.2	3	74.00
75	77	4.0	129	3	161.3	1	4000	0	0	15.09	3.87	21	7.5	3.9	1.56	0.69	42	1	166	999	0	50.1	2	32.25
76	78	4.2	79	3	161.3	1	7200	0	0	14.29	2.10	27	8.8	6.1	1.02	1.39	32	1	192	1195	0	60.9	2	19.75
77	79	12.5	73	2	142.9	2	1776	0	1	13.25	1.22	73	8.7	6.0	0.95	1.29	90	1	144	1874	0	36.2	3	18.25
78	80	19.9	187	3	157.5	2	6000	0	0	13.31	5.27	112	10.2	11.4	1.43	1.26	71	1	142	1732	0	63.3	3	46.75
79	81	6.7	84	1	156.8	1	3429	0	0	13.83	2.81	239	14.8	14.0	1.47	1.56	42	2	163	1751	0	42.6	3	21.00
80	82	17.1	115	1	160.7	1	4000	0	0	12.81	2.80	95	10.4	9.4	1.53	0.78	73	2	170	1959	0	67.0	2	28.75
81	83	6.8	149	1	165.1	1	3600	0	0	12.78	3.16	36	12.2	12.2	2.32	1.42	90	1	171	2074	0	51.8	3	37.25
82	84	10.1	288	1	153.7	2	2600	0	0	13.01	5.75	194	30.3	32.7	2.98	3.77	77	1	145	4171	0	37.2	1	72.00
83	85	10.0	112	1	151.1	1	3500	0	0	11.90	3.79	61	9.9	15.9	1.11	1.58	161	2	157	2353	0	59.1	2	28.00
84	86	31.8	243	1	161.3	1	3500	0	0	13.10	2.91	122	10.9	21.9	1.66	1.41	120	2	170	2337	0	69.8	2	60.75
85	87	6.1	268	1	161.3	1	9250	0	0	14.33	2.15	144	8.3	7.8	1.35	0.99	64	1	168	1614	0	54.8	2	67.00
86	88	9.5	99	1	157.5	2	3600	0	0	14.13	2.33	89	9.5	6.8	0.95	0.93	75	1	171	1961	0	49.6	3	24.75
87	89	17.4	188	1	154.9	2	3840	0	0	15.84	3.09	122	8.2	8.8	1.41	1.55	110	1	171	1996	0	47.9	2	47.00
88	90	.	67	1	144.1	2	2057	0	0	.	1.46	27	8.2	5.9	1.05	0.88	136	2	140	1049	0	41.9	3	16.75
89	91	16.9	124	1	156.2	2	6000	0	0	12.81	3.50	220	14.2	11.4	6.89	2.08	58	2	144	2600	0	37.2	1	31.00
90	92	8.0	365	1	158.1	1	3333	0	0	.	5.90	192	19.1	6.8	2.24	1.27	79	2	168	3676	1	50.1	3	91.25
91	93	5.1	368	1	151.8	1	11250	0	1	13.78	3.18	250	14.6	11.3	1.63	1.19	110	1	142	2119	0	40.5	3	92.00
92	94	11.1	328	1	149.2	1	8250	0	0	14.36	3.50	103	18.6	7.5	1.64	1.24	58	1	140	1832	0	43.0	2	82.00
93	95	21.8	93	1	159.4	2	2375	0	2	14.33	1.47	23	10.4	6.9	1.18	1.02	40	1	146	1431	0	74.2	3	23.25
94	96	3.2	289	1	163.2	1	4800	0	0	13.02	2.57	235	12.9	11.7	1.43	1.26	45	2	149	2513	0	54.7	2	72.25
95	97	0.5	178	1	165.1	1	6600	0	3	14.13	2.11	52	7.7	5.7	0.93	1.06	13	2	151	965	0	76.8	2	44.50
96	98	6.3	220	1	153.0	2	4750	0	0	13.43	4.48	119	17.1	19.1	2.67	2.08	71	1	146	2826	0	42.8	3	55.00
97	99	4.1	91	1	154.3	1	5333	0	0	13.17	2.59	24	11.1	9.0	1.55	0.98	73	2	170	1668	0	50.3	3	22.75
98	100	40.2	617	1	149.9	2	5000	0	0	14.31	7.02	253	29.7	16.4	3.08	2.05	118	1	140	3032	0	37.4	3	154.25
99	101	3.0	279	1	162.6	1	5000	0	0	13.89	2.69	166	9.4	6.0	1.33	2.88	50	1	170	1824	0	59.1	2	69.75
100	102	6.9	413	1	156.2	2	.	0	0	13.06	3.00	677	15.0	15.0	2.00	1.91	59	1	151	1935	0	43.7	3	103.25
101	103	3.0	129	1	149.9	2	6800	0	1	14.96	3.84	43	13.2	11.8	1.53	1.49	74	1	144	1996	0	42.3	3	32.25
102	104	6.8	232	1	160.0	1	1538	0	1	11.57	3.30	120	12.1	12.6	1.62	1.75	77	2	168	2135	0	53.9	2	58.00
103	105	18.8	204	1	167.6	2	6500	0	0	13.16	4.10	88	12.7	15.7	1.92	2.54	68	1	150	2777	0	51.2	2	51.00
104	106	3.5	111	1	148.6	1	6250	0	0	13.76	1.24	28	11.8	8.5	0.87	1.05	41	1	165	1095	0	55.1	2	27.75
105	107	3.0	345	1	152.4	2	7167	0	4	13.59	2.21	165	9.9	7.5	1.56	1.24	138	2	150	2455	0	42.0	3	86.25
106	108	39.1	95	1	157.5	2	10000	0	1	13.63	1.32	1	2.8	3.0	0.73	0.41	54	1	144	831	0	54.8	2	23.75
107	109	0.9	188	1	147.3	2	4500	0	1	13.35	2.41	62	11.6	9.2	1.42	1.20	109	1	149	2058	0	34.1	3	47.00
108	110	3.7	222	1	171.5	1	10250	0	0	14.44	4.03	49	12.9	21.0	1.90	1.78	122	1	168	2240	0	53.5	3	55.50
109	111	0.9	130	1	155.6	1	2517	0	0	13.34	2.28	67	9.4	5.0	1.54	1.27	131	2	164	2467	0	45.6	1	32.50
110	112	7.8	111	1	146.7	1	5340	0	0	14.86	3.32	28	10.7	9.1	1.39	4.42	105	1	171	2002	0	36.6	3	27.75

OBS	SUBJ	SFOL	FOL	RES	HT	MENS	PCINC	TOB	ALC	HB	VB	VC	FE	ZN	RB	MET	SUC	RACE	AGE	ENERGY	SUPP	WT	DIET	FOLRDA
111	113	0.8	172	1	157.5	1	5000	0	0	12.85	3.45	93	11.8	12.6	1.44	0.96	112	2	168	2206	0	43.7	3	43.00
112	114	4.7	90	1	158.8	2	9000	0	0	14.14	1.86	49	7.9	5.0	1.13	0.87	105	1	151	1957	0	43.0	3	22.50
113	115	17.2	148	1	147.3	2	6760	0	0	13.23	2.41	78	11.5	13.0	1.54	1.21	123	1	149	1991	0	34.8	3	37.00
114	117	6.8	700	1	153.0	2	3750	0	1	12.95	9.41	233	46.2	4.7	2.57	1.48	75	1	146	1992	1	35.3	1	175.00
115	118	11.7	40	1	163.8	1	5400	0	0	12.10	1.72	16	7.3	8.0	0.89	1.31	42	2	153	1904	0	52.5	2	10.00
116	119	3.2	391	1	153.0	2	5100	0	0	13.19	7.26	77	26.2	15.2	2.53	1.96	62	1	144	1879	1	40.0	3	97.75
117	120	9.8	187	1	160.7	1	1478	.	1	11.94	1.89	48	12.7	13.2	1.15	3.22	83	2	193	2074	0	52.2	2	46.75
118	121	0.7	114	1	157.5	1	1500	1	1	11.67	3.65	17	7.7	8.9	1.68	1.40	73	2	190	1640	0	50.7	3	28.50
119	142	0.6	105	1	158.1	1	5000	0	0	12.83	4.16	67	12.1	10.6	1.38	1.48	50	2	196	1702	0	59.4	2	26.25
120	143	16.4	582	1	166.4	1	13000	0	0	13.00	1.95	43	5.2	4.4	1.47	0.74	74	2	162	1288	0	76.1	.	145.50
121	144	2.3	166	1	161.3	1	4572	0	0	12.79	2.24	216	8.3	12.3	1.20	1.05	148	2	180	2219	0	57.5	3	41.50
122	145	23.9	164	1	161.3	1	3840	0	0	13.34	4.09	25	12.6	8.8	2.27	1.14	55	2	158	2187	0	64.1	3	41.00
123	146	20.0	190	1	151.8	1	6800	0	0	13.34	3.12	178	12.4	12.5	1.59	1.51	66	2	156	2020	0	45.5	3	47.50
124	147	7.6	82	1	159.4	2	5714	0	0	14.53	7.77	225	22.2	10.2	11.60	1.16	44	1	169	1418	0	57.3	2	20.50
125	148	2.6	233	1	163.8	1	1606	0	0	11.64	0.70	175	6.2	2.2	0.80	0.43	40	2	175	1072	0	53.8	2	58.25
126	149	8.3	289	1	161.3	1	6250	0	1	13.76	3.87	65	17.5	12.7	2.11	1.41	34	2	167	2099	0	60.4	2	72.25
127	150	6.6	426	1	157.5	1	13500	0	1	13.00	5.36	199	8.5	6.0	2.27	1.13	134	1	169	2063	1	51.6	3	106.50
128	151	0.8	422	1	167.6	1	5750	0	1	13.46	7.63	187	10.1	8.1	2.10	1.69	65	1	170	1745	1	70.5	3	105.50
129	152	6.6	118	1	153.0	1	7000	0	1	14.10	0.91	66	5 5	3.6	0.51	0.38	21	2	172	748	0	59.1	2	29.50
130	153	.	226	1	156.8	1	5730	0	0	13.89	5.22	51	12.3	11.0	1.74	1.76	105	2	177	2825	0	43.1	1	56.50
131	154	20.9	120	1	157.5	1	5833	0	0	13.55	7.47	483	24.1	5.8	13.60	0.56	42	2	173	1214	0	54.8	3	30.00
132	155	10.7	108	1	150.5	1	800	0	0	12.38	4.35	50	14.0	16.0	2.43	4.53	74	2	189	2482	0	48.3	2	27.00
133	156	22.7	208	1	167.0	1	4509	0	2	13.00	2.84	113	14.0	11.9	1.68	1.66	84	2	173	1908	0	52.1	2	52.00
134	157	0.8	252	1	158.1	1	9220	0	0	12.79	4.56	71	15.2	6.2	1.66	0.89	45	2	175	1331	0	55.0	2	63.00
135	158	17.1	292	1	168.9	1	2443	1	1	14.44	2.82	169	10.1	9.1	1.56	1.18	39	1	171	1852	0	56.5	2	73.00
136	159	4.2	136	1	165.7	1	.	1	1	14.78	1.35	39	10.9	8.5	1.07	1.19	49	2	192	1753	0	59.9	3	34.00
137	161	22.4	254	2	147.3	2	7875	0	0	13.75	3.97	130	5.8	5.5	1.66	1.14	33	1	143	1118	0	34.1	3	63.50
138	162	7.9	376	2	162.6	1	8250	0	3	14.06	2.31	29	6.6	7.9	1.14	1.05	83	1	191	1556	0	53.7	3	94.00
139	163	3.1	183	2	160.0	1	6900	0	0	14.27	2.41	79	11.5	11.8	1.28	1.44	77	1	146	1807	0	54.7	2	45 75
140	164	.	267	2	.	.	7000	.	.	.	3.75	78	9.2	2.8	1.20	0.63	78	1	150	1330	0	.	.	66.75
141	165	.	250	2	.	.	.	.	.	.	3.83	86	12.8	9.1	2.47	1.45	65	1	.	2085	0	.	.	62.50
142	166	15.6	107	3	157.5	1	8000	.	2	14.19	3.63	26	11.6	11.7	1.82	5.54	102	1	156	2375	0	1.8	2	26.75
143	167	30.1	210	3	167.6	1	5000	0	0	13.73	3.08	36	14.5	13.8	1.34	1.52	60	1	166	2007	0	48.8	1	52.50
144	168	25.2	535	2	141.0	1	3327	0	0	14.10	2.61	358	12.6	10.8	6.49	1.79	105	1	140	2099	0	44.2	2	133.75
145	169	12.2	213	2	158.8	1	8100	0	4	14.74	2.69	107	8.7	9.1	1.44	1.39	105	1	172	2349	0	48.8	3	53.25
146	170	4.9	86	3	163.2	1	4333	0	0	14.23	2.25	34	13.0	16.3	1.29	1.87	13	1	168	1638	0	44.8	3	21.50
147	171	26.0	439	3	158.1	1	5400	0	1	14.31	6.90	134	27.4	7.2	2.28	1.03	72	1	194	1844	1	57.4	2	109.75
148	172	0.8	324	2	158.1	1	7500	0	1	12.61	4.57	69	14.3	4.9	1.86	0.77	55	1	178	1481	0	69.4	3	81.00
149	173	8.9	50	3	164.5	1	5000	1	0	14.06	2.01	16	6.3	7.3	0.97	1.20	59	1	190	1330	0	56.2	2	12.50
150	174	6.9	48	2	163.2	1	4000	1	4	13.80	1.31	49	7.2	6.3	0.78	0.84	42	1	191	1205	0	48.3	3	12.00

VITA

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Candidate for the Degree of

Master of Science

Thesis: DIETARY FOLACIN AND SERUM FOLACIN IN ADOLESCENT GIRLS

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