BIOAVAILABILITY OF FOLIC ACID

FROM FROZEN ORANGE JUICE CONCENTRATE

by

© Barbara M. Rhode

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Suggested short title --

Bioavailability of Orange Juice Folate

RHODE

Dedicated to

my Mother and to the memory of my late Father,

who always inspired me to achieve my goals

ABSTRACT

B.M. Rhode M.Sc. Animal Science

BIOAVAILABILITY OF FOLIC ACID

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The effect of folate intake from orange juice on serum and erythrocyte folates was evaluated in 60 women during eleven weeks of a folate-restricted diet. Twenty-one women were users of oral contraceptive agents (OCA). Serum folate, but not erythrocyte folate, of subjects taking oral contraceptives (OCA users) was lower than in nonusers at the inception of the study ($P<0.01$). During the initial two weeks of restricted diet, serum ($P<0.002$) and erythrocyte ($P<0.0001$) folates decreased significantly. From the second to ninth week of folate-restricted diet, erythrocyte folates continued to decrease ($P<0.005$), but serum folates remained unchanged. During seven weeks of folate supplementation, one hundred ug per day of total folate activity in reconstituted frozen orange juice was as effective as 100 ug/day of synthetic folic acid in increasing serum folate ($P<0.05$), and preventing further significant decrease of erythrocyte folate. Serum folates were similar in women supplemented with folate as folic acid or orange juice. Thus the folate in reconstituted orange juice was as available to the subjects as was synthetic folic acid, and utilization of both folate forms and folate in a mixed diet was unaffected by oral contraceptive medication.

RESUME

B.M. Rhode M.Sc. Science Animale

UTILISATION BIOLOGIQUE DE L'ACIDE FOLIQUE CONTENU
DANS LE CONCENTRE DE JUS D'ORANGE CONGELE

Nous avons évalué le jus d'orange comme source nutritive d'acide folique chez 60 sujets féminins limités à une diète pauvre en acide folique pendant une période de onze semaines. Pour ceci nous avons suivi les taux sériques et intra-érythrocytaires de l'acide folique. Vingt et un des sujets utilisaient les contraceptifs oraux. Au début de l'étude, le taux sérique de l'acide folique était moindre chez les sujets utilisant les contraceptifs oraux alors que le taux intra-érythrocytaire était inaltéré ($P<0.01$). Pendant les deux premières semaines d'une diète pauvre en acide folique, les taux sériques ($P<0.002$) et intra-érythrocytaires ($P<0.0001$) de l'acide folique diminuent de façon significative. De la deuxième à la neuvième semaine d'une diète pauvre en acide folique, le taux intra-érythrocytaire continue à diminuer ($P<0.005$) mais le taux sérique demeure inchangé. Pendant sept semaines, un supplément nutritif d'acide folique (100 ug d'activité totale d'acide folique par jour) contenu dans le jus d'orange congelé reconstitué a été aussi efficace que 100 ug par jour d'acide folique synthétique à augmenter le taux sérique d'acide folique ($P<0.05$) et à prévenir une diminution ultérieure significative du taux intra-érythrocytaire de l'acide folique. Les taux

sériques de l'acide folique étaient semblables chez les sujets prenant des suppléments d'acide folique sous forme d'acide folique synthétique ou de jus d'orange. Ainsi l'acide folique contenu dans le jus d'orange reconstitué était aussi accessible aux sujets que l'acide folique synthétique. De plus l'utilisation de ces deux formes d'acide folique ainsi que l'acide folique contenu dans une diète mixte n'était pas altéré par les contraceptifs oraux.

CLAIMS TO ORIGINALITY

To the author's knowledge this research on the availability of orange juice folate was the first investigation using serum and erythrocyte folate concentrations as the indicators of absorption and utilization. Also the effect of orange juice supplementation was studied in oral contraceptive users and nonusers for the first time. This study was the first attempt to quantitate dietary folate intake in a large population of women using computer calculation of seven-day dietary surveys rather than microbiological analysis of food composites. It was felt that separate analysis of folate in foods without reported values would contribute to the literature. These analyses were performed prior to the publication of other investigations. Also this was the first known attempt to calculate the folate content of these home-prepared foods from standardized recipes.

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I. INTRODUCTION

Folate[1] deficiency may be the most prevalent vitamin deficiency in man (Herbert, 1968b; Rodriguez, 1978). The increasing incidence of low serum folate levels and megaloblastic anemia prompted the inclusion of folate for the first time in the 1968 edition of the Recommended Dietary Allowances (Food and Nutrition Board, National Research Council, 1968). Cases of folate deficiency associated with pregnancy (Lowenstein et al., 1966; Streiff and Little, 1967; Cooper et al., 1970), lactation (Metz, 1970), infancy and childhood (Baker et al., 1975; Cooper, 1976), old age (Girdwood, 1969), disease states (Weir, 1974; Shils, 1979), hospitalization (Leevy et al., 1965), drug use (Stebbins and Bertino, 1976; Roe, 1971) and alcoholism (Eichner and Hillman, 1971; Korsten and Lieber, 1979) have been reported.

Controversy exists as to whether oral contraceptive therapy results in folate deficiency. This topic has been extensively studied during recent years because of the heavy use of these drugs. In a survey completed in 1974, it was estimated that, on a worldwide basis, there were 150 to 200 million women using the preparations (Webb, 1980). Oral contraceptive agents (OCA) have been reported to lower serum and erythrocyte folate concentrations (Shojania et al., 1971; Prasad et al., 1975; Smith et al., 1975),

[1] In this thesis, the term folate refers to the group of conjugated or unconjugated compounds which owe their activity to the pteroylglutamic acid radical. The term folic acid refers to the pteroylglutamic acid itself.

leading to folate deficiency (Necheles and Snyder, 1970; Streiff, 1970; Ryser et al., 1971), but only rarely to megaloblastic anemia (Lindenbaum et al., 1975). In some cases no interaction between oral contraceptives and folate was found (McLean et al., 1969; Pritchard et al., 1971). These medications have also been implicated in altered food folate absorption (Necheles and Snyder, 1970; Streiff, 1970; Ryser et al., 1971).

Reports of folate deficiencies have emphasized the importance of obtaining accurate information on the folate content of foods. To date, evaluation of dietary folate intake has been difficult because of the lack of adequate data. Only a few studies exist listing the folate content of Canadian foods (Hoppner, 1971; Hoppner et al., 1972, 1973, 1977). Fewer reports have clearly documented the contribution of food preparation procedures to varying losses of food folates (Herbert, 1963; Leichter et al., 1978; Leichter, 1980), and eventually to folate-poor diets (Read et al., 1965; Conrad, 1970). In an effort to provide the necessary folate to humans, folate enrichment of staple foods such as maize meal and rice (Colman et al., 1975), and wheat flour and bread (Colman et al., 1975; Keagy et al., 1975) has been suggested.

The potential of orange juice for folate supplementation has not been adequately realized. Its main nutritional appeal is its content of ascorbic acid and potassium. Earlier studies (Toepfer et al., 1951; Herbert, 1963) showed oranges and the juice to contain low levels of folate. More recent reports (Hurdle et al., 1968; Streiff, 1971; Hoppner et al., 1972; Dong and Oace, 1973) indicate that orange juice, both in fresh and reconstituted frozen concentrate form, is also a rich dietary source of

folate. It can supply between 50 and 100 ug of folate in 100 to 250 ml of juice (Hill et al., 1972). Its importance as a source of dietary folate is due to its stability in the high ascorbate environment and lack of cooking (Streiff, 1971). Although orange juice may contain an appreciable quantity of folate, controversy exists about the availability of this folate.

Naturally occurring folates in orange juice exist in the monoglutamic (Streiff, 1971) and polyglutamic (Tamura et al., 1976) forms. Under physiological conditions both are probably readily available (Cooper, 1977). It would be anticipated therefore that orange juice folate should be effectively utilized. Nelson et al. (1975), using the intestinal perfusion technique, indicated that orange juice folate is equal in bioavailability to synthetic folic acid. In feeding experiments in man, however, bioavailability of folate in undiluted orange juice concentrate was low when determined by urinary excretion after a loading dose (Tamura and Stokstad, 1973; Tamura et al., 1976).

Since oral contraceptives are widely used and orange juice is a readily accessible by-product of oranges, the author sought to demonstrate that its folate is available for absorption and utilization. The major objective of the described research was to test the availability of endogenous folates in frozen reconstituted orange juice. In order to examine this subject, three subtopics were studied: (1) the hematologic folate status of OCA users; (2) the availability of folate from orange juice as compared to synthetic folic acid; and (3) the effect of orange

juice supplementation on OCA users and nonusers. In addition, the dietary folate intake and contribution by food groups was quantitated with the aid of computerized dietary surveys. Microbiological analysis of foods without reported folate values was performed.

II. LITERATURE REVIEW

The following literature review, section A, briefly describes the chemistry and physiology of folic acid and related pteridines in man; section B, discusses folate in foods with respect to microbiological assays, food composition tables, cooking and processing losses, availability, and dietary requirements; and section C, discusses the estimation of nutrient intake from individual surveys.

A. Physiology of Folate in Man

More derivatives of folic acid exist than of any other vitamin. It has been estimated that some 150 different folates could be present if the polyglutamyl side chain contained at most six residues (Rodriguez, 1978). The early history and biochemistry of folic acid and related pteridines have been reviewed by Stokstad and Koch (1967), Stokstad (1979) and Jukes (1980).

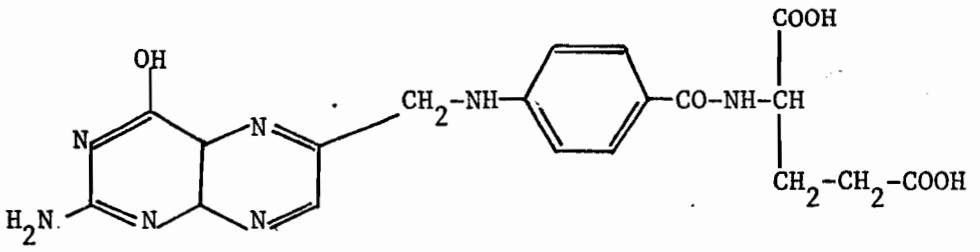


Figure 1 Folic acid (pteroylglutamic acid)

The folic acid molecule consists of three portions: a pteridine ring, para-aminobenzoic acid and glutamic acid (Figure 1). The pteridine nucleus can exist in any one of three oxidation/reduction states: folic acid or pteroylglutamic acid (oxidized); 5,6-dihydrofolic acid (partially reduced); or 5,6,7,8-tetrahydrofolic acid (reduced). The tetrahydrofolate moiety is the coenzyme form of the vitamin. Folates have single carbon substitutions at the 5-nitrogen and/or 10-nitrogen position (formyl, methyl, methylene, methenyl, formimino) of the tetrahydrofolate. The glutamate residues are linked to tetrahydrofolate and to each other by gamma peptide linkages between alpha amino groups and gamma carboxyl groups. The polyglutamates formed in this fashion are hydrolyzed to monoglutamates by conjugase enzymes found in many plant and animal tissues. In some natural products, the conjugases are associated with an inhibitor which may influence the availability of these polyglutamates (Malin, 1975; Rodriguez, 1978).

1. Metabolic role of folate

The various folic acid coenzymes act as carriers of one-carbon moieties. They are involved in the oxidation and reduction of single carbon units; in serine-glycine interconversion, methionine biosynthesis, and histidine-glutamic acid interconversion; and synthesis and catabolism of purines and pyrimidines.

Folic acid is essential for normal hematopoiesis. In folate deficiency, nucleotide synthesis is impaired. This affects DNA synthesis and leads to the development of megaloblasts (megaloblastic anemia) and hypertrophy of erythrocytes (macrocytic anemia). Folate metabolism is

also closely interrelated with that of vitamin B12 and ascorbic acid (Malin, 1975; Rodriguez, 1978).

2. Intestinal absorption of folate

Since man is totally unable to synthesize his requirements of folic acid, he is dependent upon efficient digestion and absorption of this vitamin. Dietary folates, existing primarily as polyglutamates, are absorbed in the jejunum by a process involving hydrolysis and subsequent intestinal transport of the monoglutamate form. An intestinal mucosal enzyme is required for the hydrolysis of polyglutamate to monoglutamate folate (Halsted, 1980).

Folate absorption has been estimated by measuring urinary excretion or increased serum levels after an oral dose; assessing the hematological response of a folate-deficient patient to oral doses of folic acid; perfusing a small intestinal segment and estimating the folate absorbed by the difference in concentration between the quantity infused and withdrawn; and by measuring the rise in plasma, urinary and/or fecal excretion of isotopically-labeled folate after an oral dose (Hoffbrand, 1971).

a. Absorption of synthetic polyglutamate folate

Previous studies employing microbiological assays of serum and urine were not sufficiently accurate to quantitatively determine the efficiency of utilizing polyglutamate folate. With the synthesis of the radioactive form, it became possible to quantify the digestion, absorption, and

availability of the polyglutamates. Studies in man comparing absorption of synthetic heptaglutamate folate and folic acid by urinary excretion (Tamura and Stokstad, 1973; Godwin and Rosenberg, 1975) or intestinal perfusion (Halsted et al., 1975) have demonstrated 70 to 90% absorption of the heptaglutamate as compared to folic acid. Folic acid has been used as the reference standard in most absorption studies.

b. Hydrolysis of conjugated folate

Most investigators agree that the glutamyl side chain of dietary folates must be removed or at least reduced to one glutamate residue prior to absorption (Baker et al., 1969; Butterworth et al., 1969; Godwin and Rosenberg, 1975; Halsted et al., 1975). The enzyme capable of hydrolyzing the gamma peptide bonds of polyglutamates, the gamma glutamylcarboxypeptidase or folate conjugase, has been demonstrated in many tissues. The main hydrolase activity has been associated with the intracellular lysosomes (Elsborg, 1980). Several lines of evidence point to the intestinal mucosa as the source of enzymes which deconjugate food folate (Halsted, 1980). Rosenberg and Neumann (1974) showed that the digestion of radioactively labeled polyglutamate by intestinal homogenates released the same product as that appearing in the blood after ingestion of conjugated folate.

c. Intestinal metabolism of folate

Monoglutamate folate is rapidly transported from the intestinal cells into the portal vein in man (Whitehead and Cooper, 1967; Whitehead et al., 1972). Folic acid enters portal venous blood within 10 minutes of feeding, accumulates in the liver, and appears in hepatic venous blood after an additional 10-minute delay. During this delay endogenous 5-methyltetrahydrofolate, the major monoglutamate form in man, is flushed from the liver into the hepatic venous blood system. Peak absorption occurs approximately one hour after ingestion (Whitehead and Cooper, 1967; Whitehead et al., 1972). Studies by Whitehead and Cooper (1967) and Pratt and Cooper (1971) indicated that unreduced folic acid could cross the intestine largely intact. Perry and Chanarin (1970) and Nixon and Bertino (1972) demonstrated the presence of 5-methyltetrahydrofolate in blood after feeding reduced folates. Whitehead et al. (1972) confirmed the intestinal origin of this methyltetrahydrofolate in man by demonstrating its appearance in mesenteric blood. While methyltetrahydrofolate is the principal metabolic product, there is evidence that formylfolate is also produced in the intestine (Perry and Chanarin, 1973).

d. Release of folate from epithelial cells

It is assumed that folate moves from the intestinal epithelial cells to the plasma by simple diffusion, but this assumption may be unwarranted. Folate-binding proteins may, however, play an active role in this transport (Elsborg, 1974; Leslie and Rowe, 1972). Plasma folate has been found to be loosely bound to several plasma proteins (Markkanen and Peltola, 1971),

and a small quantity is tightly held by a specific binder of unknown function (Waxman and Schreiber, 1972). Two separate mechanisms may be involved in the transportation and presentation of absorbed folates to body cells. One mechanism transports oxidized folates, and the other is selective for 5-methyltetrahydrofolate and other reduced folates (Das and Hoffbrand, 1969; Nahas et al., 1969). Intracellular folate is reduced to the tetrahydrofolate form and enters the metabolic cycle. The majority of folates is stored within human cells as the polyglutamate (Whitehead and Campbell, 1971; Noronha and Aboobaker, 1963).

3. Significance of serum and erythrocyte folate concentrations

Body stores of folate are limited to a three- to four-month supply, and signs of deficiency appear after four to six months of a severe folate-restricted diet (Herbert, 1962a). When folate ingestion is restricted, the serum level decreases within days and becomes subnormal within two weeks (Herbert, 1962a; Eichner and Hillman, 1971). Serum folate is used as an index of the dietary folate status, representing the folate consumed over the previous three to four days. Since there are continuous fluctuations of this vitamin in the diet, serum folate is not a good index of folate status.

Erythrocyte folate concentrations are not affected by minor fluctuations, and levels decrease as newer populations of erythrocytes are produced. Erythrocyte folate is an indicator of the serum folate levels available for manufacture of mature erythrocytes during the last

three months. Erythrocyte folate has been found to correlate better with megaloblastic anemia than has serum folate (Cooper and Lowenstein, 1964).

In the general population, therefore, a low erythrocyte folate is indicative of folate deficiency (Hoffbrand et al., 1966). It is probably wiser to disregard decreased serum folate levels when not accompanied by a low erythrocyte folate, although as Herbert (1962a) pointed out, the earliest evidence of a possible folate deficiency will be a decreased serum folate.

4. Factors affecting folate absorption or utilization

Many drugs have been implicated in the impairment of folate absorption and utilization. In most cases only mild folate deficiency results, but overt megaloblastic anemia may occur when drug dose is high and prolonged, or when associated with pretreatment borderline or decreased vitamin stores. Two drugs which have been reported to be associated with an altered folate metabolism are ethanol and oral contraceptive agents.

a. Ethanol

It is well known that ethanol influences folate homeostasis. The following changes are observed in chronic alcoholics: folate deficiency (Herbert et al., 1963); decreased storage of hepatic folate and diminished circulation of enterohepatic folate (Cherrick et al., 1965; Hillman et al., 1977); inhibited bone marrow response to folic acid (Sullivan and Herbert, 1964); megaloblastosis in the presence of a folate-deficient diet (Eichner and

Hillman, 1971); and malabsorption of physiological amounts of folic acid (Halsted et al., 1971, 1973; Romero et al., 1981).

In the typical alcoholic with megaloblastic anemia, serum and erythrocyte folates are decreased. Also, many alcoholics have low serum folates in the absence of morphological evidence of tissue deficiency. Well-nourished binge drinkers have been reported to have low serum folates. Since decreased folate concentrations are found in the absence of morphological changes, the serum fraction has been deemed to be of limited value as a screening test for anemia in the diagnostic workup of alcoholics. Erythrocyte folates are a better indicator of depletion and are less frequently subnormal (Lindenbaum, 1980).

b. Oral contraceptive agents

Disorders in folate metabolism may occur in women using oral contraceptives, as suggested by a number of reported observations. These have included: (1) decreased serum and erythrocyte folate concentrations; (2) megaloblastic anemia; (3) possible impairment of polyglutamate absorption; and (4) cytological changes in the cervical epithelium. This topic has been reviewed by Theuer (1972), Larsson-Cohn (1975), and Webb (1980).

Reports of the effect of oral contraceptives on serum and erythrocyte folates are conflicting. Four investigators reported significantly lower mean serum folates in OCA users than in nonusers (Shojania et al., 1971; Wertalik et al., 1972; Alperin, 1973; Smith et al., 1975), while seven other groups have not confirmed this finding (Spray, 1968; McLean et al., 1969; Maniego-Bautista and Bazzano, 1969; Kahn et al., 1970; Pritchard

et al., 1971; Stephens et al., 1972; Paine et al., 1975). Pietarinen et al. (1977) found significantly lower serum folate values in OCA users during day 5 of the menstrual cycle. Differences in dietary folate intake, selection of control subjects, sample number, assay procedures, fasting or nonfasting state, duration of OCA therapy, day of menstrual cycle at which blood samples were drawn, and individual variations in metabolic handling of folate may contribute to these discrepancies (Shojania and Hornady, 1973; Butterworth et al., 1975; Lindenbaum et al., 1975; Pietarinen et al., 1977).

Erythrocyte folates, a better index of tissue stores than serum levels, were significantly lower in OCA users than in nonusers (Shojania et al., 1971; Alperin, 1973; Prasad et al., 1975; Smith et al., 1975). Spray (1968) found that folate concentrations of OCA users and nonusers were similar. Increased urinary formiminoglutamic acid (FIGLU) excretion after a histidine load, possibly indicative of folate deficiency, has also been observed (Luhby et al., 1971; Shojania et al., 1971).

Several investigators monitored serum (Shojania et al., 1971; Smith et al., 1975) and erythrocyte (Smith et al., 1975; Ahmed et al., 1975) folate concentrations in women taking oral contraceptives from six months to four years. Both serum and erythrocyte folates decreased significantly with the length of use. Although none of the subjects developed megaloblastic anemia (Shojania et al., 1971), the number with serum folates less than 3 ng/ml rose progressively from 9% at less than one year, to 21% at two years, and 42% after four years. Pathologically low serum folate levels were reported in 2% of the controls. Shojania et al. (1971) concluded that the effect of contraceptives on folate status is mild, and in the general population, it

takes a long time for the effects to become apparent.

Cases of folate-responsive megaloblastic anemia among women taking oral contraceptives from one to five years have been reported by a number of investigators (Paton, 1969; Necheles and Snyder, 1970; Streiff, 1970; Pritchard et al., 1971; Ryser et al., 1971; Toghill and Smith, 1971; Wood et al., 1972; Alperin, 1973; Shojania and Hornady, 1973). In some instances, other underlying disorders such as malabsorption diseases, restricted dietary folate intake, and other drugs which adversely affect folate balance were present. Lindenbaum et al. (1975) pointed out that contraceptives may precipitate folate deficiency in subjects with marginal deficiency due to other causes.

Tests with women using oral contraceptives, who had not been previously saturated with folic acid, demonstrated markedly reduced polyglutamate, but not monoglutamate, absorption (Necheles and Snyder, 1970; Streiff, 1970). Stephens et al. (1972) and Shojania and Hornady (1973) found that when OCA users and nonusers were presaturated with folic acid, no difference in absorption of monoglutamates and polyglutamates was evident. Moreover, sex steroid hormones did not inhibit jejunal conjugase in vitro (Stephens et al., 1972). Streiff and Greene (1970) noted variable inhibition. Streiff (1970) had speculated that, since polyglutamates must be hydrolyzed by folate conjugase, this mechanism may be impaired by contraceptive medication. Stephens et al. (1972) and Shojania and Hornady (1973) concluded that oral contraceptives, rather than alter absorption or deplete tissue stores, may increase plasma folate clearance.

Whitehead et al. (1973) and Lindenbaum et al. (1975) observed megaloblastic cells in the cervical epithelium of women after six months of oral contraceptive use. Cytological morphology improved following three weeks of folate therapy, but abnormalities recurred three years later (Lindenbaum et al., 1975). Whitehead et al. (1973) found little relationship between these changes and hematologic findings or serum folate levels. Changes in folate-binding protein in the leukocytes and serum of women taking oral contraceptives have been reported (da Costa and Rothenberg, 1974; Eichner et al., 1975). Ross et al. (1976), however, did not observe any free serum folate-binding protein, or the presence of megaloblastic changes in the cervical epithelium of women taking contraceptives from nine to 72 months.

B. Folate Compounds in Foods

Compounds exhibiting folate activity are widely distributed in nature, being present in many animal and plant tissues. Folates are particularly abundant in dark green leafy vegetables, liver and yeast. Other good sources are nuts, green vegetables, milk, kidney, beef and whole wheat products (Damon, 1975). Oranges and their juice are also a rich dietary source of folate (Streiff, 1971). Folates exist in appreciable quantities in both the monoglutamate and polyglutamate forms (Noronha and Silverman, 1962; Butterworth et al., 1963; Santini et al., 1964; Perry, 1971; Chan et al., 1973; Dong and Oace, 1973, 1975; Shin et al., 1975). Food folates are present mostly as polyglutamates. Many of the folates in food are labile and easily destroyed by cooking, the loss being related to the amount of reducing agent in the food (Herbert and Bertino, 1967).

1. Distribution of folate forms

The distribution of folates in nature is variable, with respect to substitution in the pteridine ring and the type and proportion of polyglutamates. Kim (1970) showed the majority of plant folates to be polyglutamates containing formyl and methyl derivatives. Several reports have identified the folate pattern in foods. Folates in orange juice (Dong and Oace, 1973; Tamura and Stokstad, 1973), lettuce (Batra et al., 1973), cabbage (Chan et al., 1973), yeast (Pfiffner et al., 1946), milk (Dong and Oace, 1975; Shin et al., 1975) and liver (Noronha and Silverman, 1962; Shin et al., 1972) consist primarily of 5-methyl derivatives of

polyglutamates. Soybeans contain less than 20% of 5-methyl derivatives, and the rest is either the 5- or 10-formyl derivative (Shin et al., 1975). Schertel et al. (1965b) found that most of the folates in yeast are formyl derivatives. Fresh spinach, asparagus, endive and broccoli are comprised of 5-formylfolates (12 to 32%) and 10-formylfolates (62 to 88%). The monoglutamate is also present in broccoli (4%) and spinach (6%). No methylated forms exist in these vegetables (Santini et al., 1964). The majority of folate in beans, rice and black-eyed peas occurs as the 5-formyl (40%) and 10-formyl (46%) derivatives.

Hexaglutamate is the highest polyglutamate detected in soybeans (Shin et al., 1975). Cabbage contains mainly hexa- (30%) and heptaglutamates (50%), while soybeans contain 50% monoglutamate (Tamura et al., 1972a; Chan et al., 1973; Shin et al., 1975). The principal folate derivative in yeast is the heptaglutamate (Pfiffner et al., 1946). Orange juice is approximately 35% monoglutamate and 45% pentaglutamate (Tamura et al., 1976). Milk folate consists of 60% monoglutamate and from 4 to 8% of each of di- to heptaglutamates (Shin et al., 1975).

Several authors (Butterfield and Calloway, 1972; Hoppner et al., 1972, 1973; Santini et al., 1962; Santini and Corcino, 1974) have indicated that approximately 75% of the folates in meals contain more than two glutamates. Butterworth et al. (1963) and Santini et al. (1964) used chromatographic separation, in the absence of reducing agent, to show that an average American daily diet consists of 5-formylfolates (34%), 10-formylfolates (55%) and monoglutamates (11%). In contrast, Perry (1971) demonstrated that a mixed Western type diet contained primarily reduced methylfolates (60%),

with smaller quantities of 5-formyl derivatives (30%) and the monoglutamate (1.5%). Polyglutamates comprised 89% of the folate. Perry used ascorbate and Lactobacillus casei, as did Chanarin et al. (1968), who showed 76% of folates from meals were polyglutamates. Over half the folate analogues were methyl derivatives.

2. Microbiological assay of folate

Procedures for quantitating and separating the various forms of folate in foods involve differential microbiological assays coupled with chromatography (Butterworth et al., 1963; Herbert and Bertino, 1967). Three microorganisms have been used for determining folate presence in foods: Lactobacillus casei ATCC 7469, Streptococcus faecalis ATCC 8043 and Pediococcus cerevisiae ATCC 8081. Stokstad and Koch (1967) stated that the growth response to the folate moieties varies among these organisms. Lactobacillus casei assay measures all known oxidized and reduced pteroylglutamates containing up to three L-glutamate residues, including 5-methyltetrahydrofolate (Herbert and Bertino, 1967; Butterworth et al., 1969). To a very limited extent, Lactobacillus casei responds to folate derivatives with four to seven glutamates (Tamura et al., 1972b), but this response probably does not contribute significantly to the estimation of food folate. Streptococcus faecalis responds to folate moieties other than 5-methylfolates, while Pediococcus cerevisiae requires nonmethyl reduced folates with three or fewer glutamates (Rodriguez, 1978). Only Lactobacillus casei can utilize the majority of folate derivatives and 5-methylfolates, which are the main folates present in human serum and

liver as well as in the livers of other animals (Herbert et al., 1962).

Since many foods contain the more complex polyglutamates, their amounts cannot be estimated unless food is treated with proteolytic enzymes prior to assay. These conjugases hydrolyze the conjugated forms of folate by releasing glutamate moieties. The carboxypeptidase from hog kidney (pH 4.5) degrades polyglutamate to the monoglutamate, while the chicken pancreas extract (pH 7.8) releases a diglutamate (Cook, 1977).

Folates in food may be classified into two main groups -- free folate and total folate. The folate available to Lactobacillus casei without pretreatment with conjugase is referred to as the free folate. The total folate consists of the free folate plus those polyglutamates which are available to Lactobacillus casei only after treatment with conjugase (FAO-WHO Expert Group of the United Nations, 1970).

Many opinions have been expressed regarding the use of free or total folate for providing a better estimate of biologically available folate. Herbert (1963) suggested that free folate was the best approximation of dietary folate available for absorption and utilization by man. The Joint FAO-WHO Expert Group Report (1970) concluded that, until further knowledge was available concerning folate absorption, only the free form occurring in food should be considered. As well, the presence of polyglutamates containing more than three glutamate residues should be ignored. Tamura et al. (1972a) found that polyglutamates affect the estimation of free folate. It appeared that conjugases present in some foods cause hydrolysis of these polyglutamates during the extraction phase of the microbiological assay (Tamura et al., 1972b). Values for free folate, therefore, reflected variable amounts of polyglutamates. Also, evidence indicated that intestinal

conjugase cleaves polyglutamates, thus indicating that total folate may be more accurate (Butterworth et al., 1969; Halsted, 1979). Perloff and Butrum (1977) recommended that since the revised food composition tables contained information on total folate only, these values should be used in calculating diets. Hoppner et al. (1977) suggested that requirements be discussed in terms of total folate. The free folate fraction could be useful in assessing industrial processing losses and the efficiency of utilization.

3. Food folate data

a. Food composition tables

Depending on the choice of assay organism and whether or not conjugase is employed, extremely divergent results are obtained for the folate content of foods. Extensive data from a recent table (Perloff and Butrum, 1977) are the results of research conducted after 1963 with folate assayed using Lactobacillus casei as the test organism, and ascorbate as protection for the labile folate forms. An earlier more comprehensive table (Toepfer et al., 1951) and values from other centers (Teply et al., 1953; Burger et al., 1956; Hardinge and Crooks, 1961; Santini et al., 1962) listed folate data from foods assayed with either Lactobacillus casei or Streptococcus faecalis. However, as ascorbate protection was not used in the early work, most values were lower than those from recent studies where ascorbate was used (Herbert, 1963; Santini et al., 1964; Hurdle et al., 1968; Henderson, 1969; Hoppner, 1971; Streiff, 1971; Butterfield and Calloway, 1972; Hoppner et al.,

1972; Dong and Oace, 1973; Fung-Miller et al., 1973; Hoppner et al., 1973; Lin et al., 1975; Hoppner et al., 1977; Perloff and Butrum, 1977; Leichter et al., 1978, 1979; Klein et al., 1979; Leichter, 1980). Reported studies of the folate content of foods are shown in Table 1 (adapted from Hoppner et al., 1977). Presently the accepted procedure for folate assay is the Lactobacillus casei method, as described by Herbert (1966). The Association of Official Analytical Chemists (Horwitz et al., 1975), however, still recommends Streptococcus faecalis as the appropriate microorganism. This has resulted in some confusion in the literature.

Folate intake data from daily diets are likewise affected by values generated from assay of individual foods or composites (Chung et al., 1961; Read et al., 1965; Lowenstein et al., 1966; Van de Mark and Wright, 1972; Moscovitch and Cooper, 1973; Hoppner et al., 1977; Pietarinen et al., 1977; Elsborg and Rosenquist, 1979; Spring et al., 1979). In surveys and studies where the Toepfer et al. (1951) tables have been used, folate intake has been underestimated. Folate values were also underestimated in the tables compiled by McCance and Widdowson (1960). Moscovitch and Cooper (1973) have found that diets analyzed by newer methods contained 4.1 to 4.7 times more Lactobacillus casei folate activity than the amount calculated from the tables of Toepfer et al. (1951). Thenen (1975) therefore suggested that, until it is possible to prepare new tables, dietary folate intake should be estimated by multiplying the total folate values of Toepfer et al. (1951) by a factor of 4.4. Recent food composition tables from the United States Department of Agriculture (Posati and Orr, 1976) are based on the compilation of provisional data on the folate in selected foods from Perloff and Butrum (1977).

Table 1 Available data on folate content of foods[a]

Source	Foods reported	Remarks[b]
Toepfer et al. (1951)	200 foods - meats, fish, vegetables, fruits, cereals, dairy products - raw, processed and different market quality (compiled data 1941-1951)	FFA, TFA; L. casei, S. faecalis; chicken pancreas, no ascorbate
Tepley et al. (1953)	Various canned foods	TFA; S. faecalis
Burger et al. (1956)	30 frozen vegetables, 14 frozen fruits, 7 frozen juices	TFA; S. faecalis; chicken pancreas
Hardinge and Crooks (1961)	Compilation of various foods	TFA
Santini et al. (1962)	80 foods - meats, fish, vegetables, fruits, cereals	FFA, TFA; S. faecalis; chicken pancreas
Herbert (1963)	Various foods, raw and cooked	FFA, TFA; L. casei, S. faecalis; chicken pancreas
Santini et al. (1964)	Various foods	FFA, TFA; L. casei, S. faecalis; chicken pancreas, hog kidney
Hurdle et al. (1968)	Various foods, raw and cooked	FFA, TFA; L. casei; chicken pancreas
Henderson (1969)	Review - milk data	FFA, TFA; L. casei, S. faecalis; chicken pancreas
Hoppner (1971)	40 commercial, strained baby foods	FFA, TFA; L. casei; chicken pancreas
Streiff (1971)	Citrus and other juices	FFA, TFA; L. casei; chicken pancreas
Butterfield and Calloway (1972)	Wheat and selected foods	FFA, TFA; L. casei; hog kidney

continued

Table 1 (continued)

Source	Foods reported	Remarks[b]
Hoppner et al. (1972)	162 foods - meats, fish, vegetables, fruits, nuts, cereals, dairy products - raw, processed, and of different market quality	FFA, TFA; L. casei; chicken pancreas
Dong and Oace (1973)	Fruit juices	FFA, TFA; L. casei, S. faecalis; hog kidney
Fung-Miller et al. (1973)	Bean products: cooked, canned and instant powder	FFA, TFA; L. casei; hog kidney
Hoppner et al. (1973)	30 frozen convenience dinners	FFA, TFA; L. casei; chicken pancreas
Lin et al. (1975)	Canned garbanzo beans: effect of soaking, blanching and thermal processing	FFA, TFA; S. faecalis; chicken pancreas
Hoppner et al. (1977)	36 miscellaneous foods	FFA, TFA; L. casei; chicken pancreas
Perloff and Butrum (1977)	Compiled literature values for 299 foods	FFA, TFA; L. casei
Leichter et al. (1978)	6 vegetables: raw, cooked and their cooking water	FFA, TFA; L. casei; chicken pancreas
Leichter et al. (1979)	7 vegetables - boiling and homogenization of sample	FFA, TFA; L. casei; chicken pancreas
Klein et al. (1979)	4 vegetables - frozen, microwave and conventionally cooked	TFA; L. casei, S. faecalis; chicken pancreas
Leichter (1980)	4 canned vegetables - solid/liquid portions	FFA, TFA; L. casei; chicken pancreas

[a]Adapted from Hoppner et al. (1977)

[b]FFA, free folate activity; TFA, total folate activity

When properly interpreted, data obtained by assay with all three microorganisms, with or without conjugase digestion, can yield valuable information on the distribution of chemical forms of folate in foods. Presently folate pattern is ignored in tables of food composition, and only values based on assay with one microorganism are presented. Dong and Oace (1973) suggest that food tables should present the pattern, as well as the total amount, of food folates. Hoppner et al. (1977) point out that folate data are inadequate, and that additional information is necessary for all types of food, raw and processed.

b. Limitations of current data

Folate content of foods is greatly affected by conditions associated with processing, storage and preparation (Malin, 1975). Results of analyses of food folate content are dependent on the freshness of the food, processing procedures, manner of storage, method and length of cooking, ratio of cooking water to food and the technique of assay (Chanarin, 1969). Murphy et al. (1973) pointed out that insufficient attention has been given to the proper description and definition of samples.

Likewise, the microbiological assay of folate in foods produces large variances in food folate composition. The assay is complicated by the low concentration and multiplicity of folates, the presence of conjugase inhibitors and folate-binding fiber, and the interaction of conjugase with polyglutamates of other foods (Cooper, 1978). Other obstacles include separation of the numerous folate derivatives, and their extreme susceptibility to destruction or denaturation by heat, light, oxygen, pH and endogenous conjugase.

Recent reviewers (Herbert and Bertino, 1967; Pearson, 1967; Baker and Frank, 1968; Chanarin, 1969; Malin, 1975; Hoppner et al., 1977; Rodriguez, 1978) and researchers (Butterfield and Calloway, 1972; Dong and Oace, 1973; Malin, 1974; Hoppner and Lampi, 1977) have stated that numerous factors may affect the microbiological assay. Some examples are listed below: the microorganism used, the standard, source and purity of conjugase, extent of deconjugation of the polyglutamates, the presence of inhibitors or stimulants other than folate, extraction methods, use of filtration, presence and strength of reducing agents, pH of incubation and incubation time. Rothenberg et al. (1972) found that the assay itself is subject to variability. Butterfield and Calloway (1972) reported that the coefficient of variation for the Lactobacillus casei assay was approximately 25%.

4. Factors influencing the stability of food folate

Data relating to the effect of cooking and processing on folate in foods are limited and controversial. Authors agree, however, that decreases of folate in cooked and processed foods result from both destruction and leaching into the cooking water (Herbert, 1963; Taguchi et al., 1972, 1973; Fung-Miller et al., 1973; Lin et al., 1975; DeRitter, 1976; Leichter et al., 1978). Studies show that almost always foods are lower in folate after cooking and that the magnitude of loss varies greatly among foods. This is possibly due to the differences in the stability among folate forms and in the assay methodology (Cooper et al., 1978).

a. Losses due to cooking

Cheldelin et al. (1943) studied the effect of cooking foods and concluded that, of all vitamins tested, folates were the most susceptible to destruction. With the exception of liver and sauerkraut, losses in selected foods ranged from 46 to 97%. Schweigert et al. (1946) demonstrated that 10 to 46% of folate was retained in meat after roasting, braising and stewing. Hanning and Mitts (1949) found folate losses of 18 to 48% when boiling, frying or scrambling eggs. Herbert (1963) suggested that the majority of folate may be lost through boiling of foods in large quantities of water. Herbert (1968a) also estimated that up to 95% of the initial folate in foods could be lost by oxidative heating processes, and that the loss was greater when more of the surface area of the foods was exposed. Presently loss of folate from vegetables during cooking is believed to be caused principally by extraction into the cooking water rather than by destruction (Fung-Miller et al., 1973; Leichter et al., 1978). Nutrient losses tended to increase as the ratio of cooking water to food increased.

Decrease in free folate appeared to be greater than that of total folate during cooking. Taguchi et al. (1973) measured the loss of folate in 19 foods after boiling, and showed a decrease in both free (range, 50 to 90%) and total (range, 10 to 80%) folate after 5 minutes. As much as 95% free folate and 80% total folate was lost after 15 minutes boiling. A mean loss of 73% for free folate and 45% for total folate was found for cooked foods (Huskisson and Retief, 1970). Leichter et al. (1978), however, disagreed that the large decreases in free folate were due to its thermal

instability. They believed that free folate in cooked vegetables probably represented a more accurate measure of the actual folate content than it did in the raw, which was biased by an artificial increase. The higher free folate values in raw vegetables could be due to the action of naturally occurring conjugase on folate polyglutamates during the extraction phase of the assay (Tamura et al., 1972a; Leichter et al., 1979). Reed et al. (1976) and Stokstad et al. (1977) similarly observed deconjugation of the polyglutamates in raw chicken liver and meat during prolonged storage or folate extraction.

b. Losses due to processing

Suckewer et al. (1970) reported variable losses of free folate and increases in total folate when processing canned French beans and green peas. Also 60% of the initial free and total folate content of tomato juice was destroyed with processing. Differences in folate losses were attributed to the amount of exposure to temperature and oxygen on the production line. Leichter (1980) concluded that little folate destruction occurs during the canning process. The losses of folate that seem to occur during canning are due to leaching out from the vegetables into the liquid portion. Canned pinto beans were also found (Fung-Miller et al., 1973) to be more susceptible to loss from leaching than to destruction by heat. A study of the folate in canned chickpeas (Lin et al., 1975) showed that most of the loss occurred during the blanching prior to the actual heat treatment of the canning process.

The packaging material had an important effect on folate content. Storage for 12 months in dark glass bottles produced a 7% decrease in folate, while clear glass caused a 30% loss (Suckewer et al., 1970). Krehl and Cowgill (1950) noticed little change in the folate content of citrus juices during canning and storage for 6 months. Hardinge and Crooks (1961) observed a decline of folate in fresh fruit juice on canning. Hellendoorn et al. (1971) reported stability of folate in canned pulses, potatoes and meats after sterilization and storage for 1.5, 3 and 5 years.

Henderson (1969) reviewed the literature on the effects of ultra high temperature (UHT) processing and subsequent storage on the folate content of milk. Oxygen caused a loss of 50% in folates on heating and up to 80% on further exposure to sunlight. Deaeration of milk before processing effectively decreased folate loss, as did the presence of ascorbate in milk.

Wartanowicz and Rakowska (1974) observed a 7 to 60% loss of free folate and 11 to 80% total folate loss during the processing of cereals. The degree of loss was dependent on the method and grade of grinding. Calhoun et al. (1958) and Schroeder (1971) demonstrated a significant decrease in folate associated with the milling and refining of flour. In India it was found that breads made from high extraction rate flours were a rich source of dietary folate (Dutta et al., 1980). Fermentation of dough produced a further decrease in total folate content (Suckewer and Secomska, 1971), as did the baking procedure. Keagy et al. (1975) stated that the folate content of bread depended largely on the fermentation time, and whether yeast or baking powder were used as starters.

The effects of other processes, such as freezing, drying and microwave heating, are being gradually studied. Freezing and thawing appear to have a similar effect on food polyglutamates as do the conjugases (Cook, 1974). Hoppner et al. (1973) showed that the total folate of frozen reheated dinners did not differ significantly from values obtained before reheating thawed meals. Free folate levels were considerably affected, with a mean loss of 22% after reheating. Klein et al. (1979) found that the folate content of microwave and conventionally cooked vegetables was not significantly different.

5. Availability of food folate

a. Individual folate compounds

Considerable uncertainty exists concerning the availability of the various folate forms for absorption and utilization. It is widely assumed that the monoglutamates are readily available for absorption, as is the folic acid itself. In a group of 13 control subjects, 79% of a 200 ug test dose of tritiated folic acid was found to be absorbed (Anderson et al., 1960). Girdwood (1953) stated that the 5-formyl forms of reduced folates may be destroyed during ingestion by the acidic gastric juice, although Butterworth (1968) observed clinical improvement and reticulocyte responses in one patient with tropical sprue fed 50 ug folinic acid daily. Oral daily administration of 100 ug synthetic 10-formylfolates in tropical sprue produced responses in four out of nine patients. Feeding of formylfolates was effective in the therapy of megaloblastic anemia of pregnancy, and in two other patients using lettuce, and soups prepared with lettuce, spinach and asparagus

(Butterworth, 1968). Tamura and Stokstad (1973) stated that the 5-formyl and 5-methylfolates, and synthetic tri- and heptaglutamates had about the same availability as folic acid.

b. Yeast folate

Polyglutamates account for 95 to 97% of the folate activity in yeast (Schertel et al., 1965b; Butterfield and Calloway, 1972), with the principal folate form being the heptaglutamate (Pfiffner et al., 1946). Reports of the availability of the heptaglutamates in yeast and yeast extracts are variable, and are influenced by the technique of preparing the extract, the status of the individual, the quantity consumed and the criteria used. Bethell et al. (1947) found little response in patients with pernicious anemia upon administration of a crude yeast concentrate. Patients responded hematologically when given purified yeast conjugate, and urinary excretions were similar to those produced by an equivalent amount of folic acid activity. Swendseid et al. (1947) suggested that yeast contained a conjugase inhibitor, since adding crude yeast extract to the purified concentrate decreased urinary folate activity to 15% of the amount excreted when subjects ingested folic acid. It appeared that more conjugase was needed to split the polyglutamate in the crude preparation. Crude yeast extract, however, had no effect on the absorption of folic acid.

Several studies have been reported on the availability of folate in yeast and its extract (Jandl and Lear, 1956; Schertel et al., 1965a; Perry and Chanarin, 1968; Tamura and Stokstad, 1973; Grossowicz et al., 1975; Babu and Srikantia, 1976). From a yeast extract containing 90%

polyglutamates and 10% free folate, only 25% of the folate activity was absorbed by two subjects. When the preparation was treated with conjugase, however, 60% of the test dose was absorbed as compared to 95% of the folic acid (Jandl and Lear, 1956). Schertel et al. (1965a) showed that 22 to 31% of folates were available from dried brewer's yeast, and that this figure diminished to 8% when yeast extracts were tested. A change in erythrocyte folate was observed in two groups of healthy subjects given a daily oral supplement equivalent to 100 ug free folate, as either the folic acid itself or 17 yeast tablets (Perry and Chanarin, 1968). After eight weeks the erythrocyte folates were 45% above baseline in the group receiving folic acid versus a 15% rise for those receiving the 100 ug of heptaglutamate. Perry and Chanarin (1968) concluded that the yeast extract folate was one-third as available as an equal amount of folic acid. Tamura and Stokstad (1973) reported 60% absorption of folate from yeast, while Babu and Srikantia (1976) showed a mean availability of 10.1%. Subjects from both studies were presaturated with folic acid, and availability was calculated from a dose-response curve. Grossowicz et al. (1975) noted that small amounts of yeast folate fed to healthy subjects were fully utilized. Intakes of 300 ug folate from the extract produced similar elevations in serum folates as did 300 ug folic acid. A ten-fold increase in folate intake from the yeast extract, however, did not produce an increment in serum levels. It appears that while folate from a pure conjugate of yeast may be utilized as well as folic acid, it has low availability in the presence of conjugase inhibitors which are present in yeast.

c. Orange juice folate

Controversy exists regarding the availability of folate from orange juice. Streiff (1971) stated that both the fresh and frozen concentrate contain an appreciable amount of the monoglutamate, and this form is easily absorbed. Tamura and Stokstad (1973) found that folate from frozen concentrated orange juice was only 31% available. They later reaffirmed the poor availability of folate (Tamura et al., 1976). Orange juice also inhibited the availability of added heptaglutamate (Tamura and Stokstad, 1973; Tamura et al., 1976).

Tamura and Stokstad (1973) used a method in which folate absorption from food was estimated from the subsequent urinary excretion of folate. Human subjects were maintained in a saturated state by oral administration of a 20 mg dose of folic acid initially and then 2 mg on alternate days. The test foods and synthetic folates were given every other day. Dose-response curves relating the oral dose with urinary folate excretion were established. Folates which were absorbed were then estimated with reference to these response curves. An index of the availability of folate derivatives and folates in natural foods could then be calculated. Experiments were performed to test for an inhibitory factor in those foods which demonstrated low folate availability. The percent availability of folates was computed by comparing the quantity of folate absorbed with the amount of total folate in the test foods. Estimation of the availability of the folic acid or heptaglutamate given with orange juice was also made.

The reported low availability of folate from 600 ml of concentrated frozen orange juice (2400 ml fresh juice) is puzzling. The presence of a low pH was not considered to be an explanation, since absorption was unchanged by correcting the pH with sodium hydroxide. Tamura and Stokstad (1973) postulated that other inhibitory factors may exist in orange juice which impede the absorption of its folate. Also the large volume given was not representative of a typical intake. Tamura et al. (1976) prepared a synthetic orange juice containing citric acid, malic acid, ascorbic acid, glucose and sucrose in the proportions available in 600 ml of orange juice concentrate. They showed that the heptaglutamate was only 54% available when given with orange juice, but increased to 85 and 84% when administered with the synthetic juice or citrate plus sugars, respectively. They concluded that the low availability of the heptaglutamate was due to the inhibition of intestinal conjugase caused by the low pH (pH 3.7). They reasoned that the pH could be an important aspect in the absorption of polyglutamates.

Nelson et al. (1975) employed the triple lumen intestinal perfusion technique to relate absorption of folate from reconstituted orange juice to that from a synthetic mixture. Orange juice folate was found to be as available as the folic acid. Approximately 60 and 58%, respectively, were absorbed during triple lumen perfusion. Colman and Herbert (1976) have, however, questioned the design of their experiment. Nelson et al. (1978) further demonstrated that the drug diphenylhydantoin did not affect the absorption of folate from reconstituted orange juice.

d. Dietary folate in other foods

The availability of folate from foods has been investigated by comparing urinary excretion of folate from various foods to individual response curves established with folic acid (Retief, 1969; Tamura and Stokstad, 1973; Babu and Srikantia, 1976). Retief (1969) was unable to control fluctuations in the urinary folate excretion, but Tamura and Stokstad (1973) and Babu and Srikantia (1976) overcame this problem by saturating the subjects with folic acid during the test period. Table 2 illustrates the results from the latter two studies (adapted from Rodriguez, 1978).

Retief (1969) reported that the availability of folate from liver, spinach and peas was high, but that in cauliflower, lettuce, pumpkin and tomato was low. Babu and Srikantia (1976) and Tamura and Stokstad (1973) noted 70 and 50% availability, respectively, from liver. More than 50% availability of folate from six commonly used foods was observed (Babu and Srikantia, 1976). The availability of cow's milk folate was found to be identical to that of folic acid in two infants with megaloglastic anemia (Ghitis and Tripathy, 1970). Tamura and Stokstad (1973) observed low availability of folatés from romaine lettuce (25%), wheat germ (30%) and egg yolk (39%). Higher availability was found with bananas (82%), dried cooked lima beans (70%) and frozen cooked lima beans (96%).

It appears that the biological availability of folates varies widely with each food and subject studied. Differences exist among individuals in their ability to absorb folate from foods, especially with substances of a low folate content. The variation in folate availability ranged between

Table 2 Availability of folate in foods[a]

Food	Total food folate in supplement (ug)	Basal dose of folic acid (ug)	Total folate in supplement (ug)	Availability Mean (%)	Availability Range (%)
Banana[b]	192-252	400	592-652	45.6	0-148
Banana[c]	250	350	600	82	0-148
Bengal gram[b]	282	400	682	68.8	29-163
Cabbage (cooked)[c]	330-430	200-350	530-780	47	0-127
Cabbage (raw)[c]	490	200-350	690-820	47	0-93
Defatted soybean meal[c]	610	350	910	46	0-83
Egg (hen)[b]	210-350	400	610-750	72.4	35-137
Egg yolk[c]	350	400	750	39	0-129
Green gram[b]	314	400	714	55.2	0-118
Lima beans (dry, cooked)[c]	240	350	590	70	0-138
Lima beans (frozen, cooked)[c]	420	350	770	96	48-181
Liver (beef, cooked)[c]	670-1010	0-350	670-1360	50	22-103
Liver (goat)[b]	315	400	715	70	9-125
Orange juice[c]	840	none	840	31	17-40
Romaine lettuce[c]	750	none	750	25	12-37
Spinach[b]	310	400	710	62.8	26-99
Tomato[b]	300	400	700	37.2	24-71

continued

Table 2 (continued)

Food	Total food folate in supplement (ug)	Basal dose of folic acid (ug)	Total folate in supplement (ug)	Availability Mean (%)	Availability Range (%)
Wheat germ[c]	730	350	1080	30	0-64
Yeast (Brewer's)[b]	300	400	700	10.1	0-36
Yeast (Brewer's)[c]	1400	none	1400	60	55-67
Yeast extract[c]	750	none	750	63	59-69

[a]Adapted from Rodriguez (1978)

[b]Values from Babu and Srikantia (1976)

[c]Values from Tamura and Stokstad (1973)

0 and 148% (Tamura and Stokstad, 1973; Babu and Srikantia, 1976). Values greater than 100% were difficult to explain. Tamura and Stokstad (1973) pointed out that large amounts of a particular food may not depict the absorption pattern when more normal quantities are ingested. Babu and Srikantia (1976) found the free folate fraction to be a poor indicator of the availability of total folate.

6. Human adult folate requirements

The amount of folic acid needed by man is not accurately known. The assay of folate in foods is still unsatisfactory, and complicated by the various forms of folate compounds. The problem of availability for absorption also complicates the picture.

Folate deficiency has been produced experimentally in man by a diet furnishing 5 ug of folate daily (Herbert, 1962a,b). It has been suggested (Zalusky and Herbert, 1961; Herbert, 1962b) that the minimum requirement may be between 50 and 100 ug/day. However, folate deficiency was not induced with diets containing less than 8 ug daily (Velez et al., 1966).

In view of the many uncertainties, the FAO-WHO Expert Group of the United Nations (1970) recommended a dietary intake of 200 ug free folate per day for healthy adults. They indicated that requirements would be increased during periods of rapid growth. Pregnancy would increase allowances by 2 to 4 times. This group suggested a daily dietary intake of 400 ug free folate throughout pregnancy and 300 ug during lactation.

The Food and Nutrition Board of the National Research Council (1968) first established a Recommended Dietary Allowance (RDA) for folate, and since then requirements are given in terms of Lactobacillus casei activity. The 1974 edition of the RDA for folate gave 400 ug for adults, and 800 and 600 ug during pregnancy and lactation, respectively. Folate allowances published recently (Food and Nutrition Board, National Research Council, 1980) refer to total folate levels. The requirement remains unchanged for adults.

The Canadian Recommended Daily Nutrient Intake (RDNI) listed a requirement of 200 ug of free folate for adults (Health and Welfare Canada, 1975). Cooper (1978) suggested that the recommended daily intake of folate from the diet for adults is approximately 150 ug per day of total folate. Rodriguez (1978) reviewed the issue of human folate requirements extensively.

C. Estimating the Nutrient Intake From Individual Surveys

1. Introduction

The collection of food intake data is an essential part of dietary surveys conducted for nutritional, medical and economic purposes. The aim of these surveys is the measurement and evaluation of the nutrient intake and food consumption of populations, population groups or individuals.

No single approach exists which is unanimously accepted for appraising the dietary intake of individuals. Experience with the collection of data on dietary intake has been extensive, and many methods for observing and recording food intake and of obtaining dietary histories have been used. The weaknesses and strengths of different methods are well known to most investigators, despite limited statistical data on the reliability and validity of the various methods. No single approach seems adequate for all situations. Rather selection is determined by the precise objectives of the study (Marr, 1971; Pekkarinen, 1970).

2. Factors affecting the choice of methodology

The choice of methodology is usually a compromise between several factors -- the aim of the survey, the sample size required, and the funds and number of qualified personnel available. The objective of the survey is the primary factor controlling the choice of method. It specifies the accuracy of the consumption data needed. The second most

important factor is the sample size. In many cases a compromise between sample size and accuracy is required. The variability of the diet must also be considered in selecting the time span to be evaluated. Lastly, costs dictate the choice of a method capable of dealing with a sufficiently large sample at the risk of affecting the reliability of the results (Pekkarinen, 1970).

3. Survey methods for measuring individual food consumption

Dietary surveys are designed to measure current food consumption and assess food habits over a specified period. The principal methods used to collect such data include (1) RECORDS of current food intake -- (a) the weighed record method and (b) the estimated record method, (2) RECALLS of past food intake -- (a) 24-hour recall method, (b) dietary history method, and (c) frequency method, and (3) a COMBINATION of the two principal methods -- combined recall-record method (Marr, 1971).

a. Record of current food intake: Weighed record method

The weighed record is the commonly accepted measure against which other measures of individual diets are validated. It is the best survey procedure for yielding the most precise measure of the food eaten. A weighed record is a listing of all foods consumed by an individual during a specified period with the amounts of each, determined by weighing and entered in grams. The prepared food portions, either in the raw or cooked form, are weighed before serving and any discards are accounted for.

Nutrient content is generally calculated using food composition tables in which cooked foods and home-prepared items are included. All foodstuffs appear as edible amounts. When foods are eaten outside the home, portion sizes are assessed in household measures or by estimation (Becker et al., 1960; Marr, 1971; Pekkarinen, 1970). This weighed record procedure provides the most accurate measurement of actual food consumption, but serious disadvantages are reported in the literature.

The size of the sample is usually small because of the time and skill required of the investigators. Pekkarinen (1970) wrote, "It is seldom representative. Random sampling cannot be employed, and volunteers are selected. The results thus obtained cannot be generalized." She concluded, "Despite its many limitations the weighing method has been used in a large number of surveys, especially in such surveys where the accuracy of the data (i.e. physical measurement of foods ingested) is more important than the size of the sample. For this reason it is restricted to relatively small surveys...."

The validity of data obtained by this method is questionable on other grounds. Most reports of studies involving weighing procedures remark on the problem of changes in food patterns because of the burden of measuring (Pekkarinen, 1970). The usual reaction is to simplify the diet, although sometimes attempts are made to impress the investigator with particular dietary practices. Ohlson et al. (1950) commented that snacking became inconvenient when it was necessary to weigh each mouthful, and snacks were seldom found on the weighed diet records. Another research report based on estimated records indicated that some individuals

were embarrassed to reveal all that they really had eaten (Paul et al., 1963).

Time considerations are based essentially on the fluctuations of an individual's dietary intake over various periods of time. Reliability of the weighed record is very dependent on the length of the recording period, and on the comparison of consumption between time periods. Evidence of actual day-to-day variation in food and nutrient intakes is provided by Wait and Roberts (1932). They noted the following factors as producing the day-to-day differences in the kinds of foods eaten: (1) Previous day's intake, with the lowest intake often following the largest, (2) serving of food liked or disliked, (3) physical activity, (4) variations in economic stress, (5) emotional state, (6) variability in health status, and (7) festive days.

The dietary record should cover a sufficient period of time to furnish an adequate picture of nutrient intake, and also avoid loss of interest and cooperation. The minimum time is usually one week, when most of the daily variations in the diet become apparent. Weekly and seasonal variations must also be considered. Some investigators have revealed that weekly variations may be an important feature in dietary surveys. For Yudkin's study (1951), six young dietitians weighed and recorded their daily intakes during four consecutive weeks. Food energy, protein and fat averaged 10 to 25% higher for the first week of diets than for the following weeks. Vitamin A consumption was two to five times greater in one week compared with another. Yudkin concluded from these six case studies that one week was too short a period to assess an

individual's intake accurately. Chappell (1955) also considered seven days to be too short a period to accurately assess the intake of nutrients by an individual. Still it is not practical to use longer survey periods than one week at a time. It is more beneficial to carry out several one-week surveys in different seasons.

Even shorter recording periods than seven days have been successfully employed. Eppright et al. (1952) found that three weekdays did not give the same indications of dietary adequacy as one day or seven days. In comparing distributions of one-day dietary records with three-day dietary records and three-day with seven-day records, the one-day diets appeared to be more adequate than those over three days, and the diets for three days in turn appeared more adequate than those from seven-day diet records. Chalmers et al. (1952) concluded that "a dietary record need consist of only one day when characterizing the dietary intake of a group", and it is more effective to increase the number of subjects than the number of days. However, some researchers found certain combinations of days seemed to affect the measurement of nutrient intakes. Cellier and Hankin (1963) and Eppright et al. (1952) found dietary intake on weekend days often differed from intakes on weekdays. The weekend days were thus important to include. Less important was which of the weekdays was included.

b. Record of current food intake: Estimated record method

The estimated record procedure is often called the household measure record because standard household measuring utensils, the eight-fluid ounce measuring cup and measuring spoons, are used for quantitating the amounts of each food eaten. If a food item cannot be measured volumetrically, it may be measured with a ruler and dimensions listed, as for a piece of meat or cake. For some items counts are used, such as the number of eggs or slices of bread. For candy bars or prepackaged portions, weights are readily available and are used. When no relevant measuring device is available, the portion size is estimated or described as accurately as possible. Accordingly several kinds of measurements, varying in their precision, usually appear on the estimated records (Becker et al., 1960; Pekkarinen, 1970).

Low cooperation rates and poor representativeness of the samples are two features of the estimated record method. Marr (1971) stated that subjects using records in surveys did not necessarily cooperate more than those in a weighed survey, yet the record survey was regarded as eliciting the better response.

Two elements enter into comparisons of data from estimated records with those from weighed records. One is the reduction of precision arising from use of various volumetric measures, instead of weights measured with scales. Additional inaccuracy arises from conversion of household measures to weights. The other is possible differences between servings weighed beforehand by experienced adults and those estimated in

volumetric measures by consumers (Pekkarinen, 1970).

Studies by Bransby et al. (1948) and Eppright et al. (1952) indicate some significant differences in precision of measurement. The Eppright study included records of 25 children's intakes that were estimated and then weighed by their mothers. The researchers found nutritive values calculated from the two types of records to be correlated, but those from estimated records were consistently higher. They concluded that when records are being kept by untrained people, the information on food items was probably as satisfactorily obtained by servings as by weighed amounts.

These findings indicate the basic problem of estimating portion sizes which are inherent to both the estimated record and the recall procedures. Some researchers have used plastic measures and shapes to help respondents evaluate sizes and amounts of portions difficult to estimate (Becker et al., 1960; Pekkarinen, 1970). Others have used food models and concluded that they increased the accuracy of portion sizes (Moore et al., 1967).

Accuracy of the estimated record may be affected by changes in consumption brought about by participation in the survey. Young et al. (1953) showed that collection of dietary information altered the subjects' food intake systematically so that the 18 young adults recorded higher mean caloric and nutrient intakes for the first week than in the succeeding three weeks except for vitamin A.

4. Use of food composition tables

Another source of error associated with the dietary surveys is the inconsistency in precision of the steps taken in collecting and analyzing dietary data. In several studies after obtaining precisely weighed records of food quantities, the nutrient contents were obtained partly from precise chemical analyses of some foods and partly with imprecise shortcut methods, such as applying one value for a group of foods (Fry et al., 1963; Leverton, 1937). Unless the diets of the subjects contain food items in the same proportions within food groups as in the basic data, there is a possibility of introducing bias. The average food composition values from tables, such as in Agriculture Handbook No. 8 (Watt and Merrill, 1963), are likely to result in less error in nutrient estimates than when based on samples, since the handbook data are adjusted for variations in nutrient content among seasons and areas, as well as varieties (Murphy et al., 1973). Young et al. (1952) reported that the method of calculation from food composition tables was sufficiently accurate for group averages and thus could be used for survey purposes.

Bransby et al. (1948) reported good association between the calculated and analyzed values for calories and those nutrients listed, except for iron. The calculated values for calories, carbohydrate and fat tended to be higher, and for protein, calcium and iron lower than the analyzed values. Whiting and Leverton (1960) showed good correlation between figures for analyzed and calculated protein and caloric content of diets. Calculated values for fat tended to be considerably higher than

analyzed results, because table values for fat were based on carcass cuts of meat rather than trimmed retail cuts. Other researchers have also found reasonable agreement between calculated and analyzed values, although constant advancements in food processing techniques may account for some of the discrepancies (Manalo and Jones, 1966; Monsen et al., 1967).

5. Conclusion

In general it must be concluded that, on an individual basis, results to be obtained from one method cannot be predicted by another method. With differing methodologies, different factors are being measured. Though comparisons of one procedure with another have been made, these comparisons are between methods whose accuracy and reliability are not known. Therefore no conclusions may be reached regarding which method appears to give the most valid index of what it is about dietary intake that should correlate with various aspects of the problem being studied. The best method depends on the objective of the study and the hypothesis to be tested.

III. MATERIALS AND METHODS

A. Subjects Studied

1. Introduction

Sixty healthy Caucasian female volunteers participated in this investigation over an eleven-week period, from the end of September to mid-December, 1977. Subjects were McGill University undergraduate and graduate students, dietitians and dietetic interns, and women employed in clerical and other professional positions at three Montreal hospitals. Thirty-six participants had at least two years dietetic training. All women were of similar socioeconomic status.

These subjects were selected on the basis of the following criteria: (1) good health and a nonpregnant state and (2) use and nonuse of combination type oral contraceptives.

2. Recruitment

Volunteers were recruited from McGill University and the three hospitals through contact with senior personnel from various departments. Meetings were initially scheduled with dietetic interns and dietitians. Letters outlining the study were sent to Internship Directors prior to the meetings. Contact was also made with the School of Nursing, senior hospital nursing and clerical staff, and the School of Food Science. All were asked to present the study to their students and/or employees. Additional

participants were attracted through an advertisement placed for three consecutive days in the university campus newspaper. A meeting was scheduled with those women who were interested in participating.

During orientation with groups or individuals, the purpose and nature of the clinical trial were explicitly described. On admission to the study, all subjects received a letter restating the points discussed and answered a questionnaire. Data regarding demographic information, food store preferences and vitamin and oral contraceptive ingestion were collected (Appendix Table Ai). All participants received a calendar listing dates and research events. An informed consent statement was signed at the first blood sampling (Appendix Table Aiv). A $25.00 cash reward was given on successful completion of the study.

3. Experimental design

Volunteers were divided into two groups on the basis of using oral contraceptives. Of the 60 subjects, 21 women (aged 18 to 33 years, mean 23.6 years) had used combination type oral contraceptive agents (OCA users) for at least six consecutive months prior to participating in this study. Use of contraceptives ranged from six months to nine years. The oral contraceptives taken and the number of subjects using particular brands were as indicated: Ortho-Novum 1/50 (6 subjects), Ovral (5), Min-Ovral (4), Norinyl-1 (2), Logest 1.5/30 (2), Minestrin 1/20 (1) and Brevicon (1). The second group consisted of 39 women (aged 20 to 45 years, mean 23.8) who had either not previously been on oral contraceptive therapy or had

discontinued their use for at least six months before this investigation. This group was designated as nonusers. None of the subjects had been pregnant during the preceding six months, and none were lactating. All ate usual mixed diets and were within ± 20% of their ideal body weight (Metropolitan Life Insurance Company, 1959). Use of any drugs except aspirin and oral contraceptives was not permitted. The intake of aspirin was not quantitated. Vitamin supplementation was discontinued on commencement of the study. Only 8.3% of the volunteer subjects had previously ingested vitamin supplements, five percent of whom were OCA users. None of the vitamin preparations contained folic acid. Subjects were asked to limit alcohol intake to 240 ml/day. Alcohol consumption was recorded during two periods -- (1) week 5 to 9 and (2) week 9 to 11. All subjects completed at least two menstrual cycles, and all 21 OCA users were taking contraceptives at the times of blood sampling.

B. Experimental Protocol

1. Blood sampling

Blood samples were collected by venepuncture from fasting subjects using the Vacutainer Blood Collection System[1] before (week 0) and at 2, 5, 9 and 11 weeks after the commencement of the study. Blood was secured from the same subject on the same day of each sampling week.

A 4.5 ml aliquot of citrated blood was used for the determination of erythrocyte folate. Of this, a 0.1 ml whole blood sample was hemolyzed in 1% ascorbic acid and frozen at -20°C for erythrocyte analysis. Ten ml of clotted blood were obtained and serum used for the determination of serum folate. Samples were stored at -20°C for less than one week before assay. Serum and erythrocyte folates were measured microbiologically with Lactobacillus casei (American Type Culture Collection)[2] ATCC 7469, using a slight modification (Cooper, 1973) of standard assay procedures (Baker et al., 1959; Herbert, 1966). Concentrations were reported as ng/ml. Each assay was completed in triplicate and pooled sera were used as a control. The coefficient of variation between assays was 15%.

The Pincourt Medical Center (Lakeshore Diagnostic Services, Inc.) and the Vitamin Laboratory (Royal Victoria Hospital) were the centers

[1]Becton, Dickinson and Company Canada, Mississauga, Ontario

[2]American Type Culture Collection, 12301 Parklawn Dr., Rockville, Maryland

used for blood sampling. Blood was drawn by the same technician in each of the two centers. The author was present at all blood sampling sessions, checking attendance and labeling blood tubes. A printout reporting serum and erythrocyte folates at the weeks of blood sampling, with an explanation of the clinical significance, was sent to all subjects at the end of the study (Appendix Table Av).

2. Dietary restriction

All 60 subjects were instructed to take a folate-restricted diet for the entire eleven weeks of the study. This diet excluded foods high in folate content including liver, kidney and spinach, as well as oranges, tangerines and orange juice, products whose folate was under investigation. Eggs were limited to three per week because of the high folate concentration in the yolk. Home-prepared foods and mixed dishes were permitted. The folate-restricted diet was self-selected and self-prepared.

At the blood sampling sessions, in addition to the collection of blood samples and distribution of the orange juice and folic acid supplements, subjects were interviewed with attention paid to dietary adherence and to the provision of support and instruction in maintaining the folate-restricted diet. The subjects' dietary habits were reviewed by a simplified oral diet history.

3. Folate supplementation

All 60 women adhered to the folate-restricted diet during a two-week baseline period during which no supplements were administered. Following the second blood sampling at week 2, subjects were randomly assigned to three treatment groups for the next nine weeks using a table of random numbers. Treatment groups consisted of no folate supplementation (control or nonfolate supplemented group), daily supplements of a pool of reconstituted orange juice concentrate (orange juice group) and daily supplements of 100 ug of synthetic folic acid in water (folic acid group). Nineteen women were assigned to the control group, 21 to the orange juice group, and 20 to the folic acid group.

Orange juice supplementation was with a single batch of commercial Florida frozen concentrated orange juice product (orange juice). Supplies of frozen concentrate were provided in quantity, and were stored at -20°C. Each subject reconstituted single cans of this concentrate (1:3 dilution v/v), and stored the fresh product in an amber jar in the refrigerator at home. Fresh solutions were prepared every third day. Free and total folate content of the freshly prepared orange juice was 19.4 and 34.8 ug/100 ml, respectively. Folic acid supplementation was with synthetic folic acid solution (Folvite)[1] diluted 1:50 (v/v) in distilled water in the laboratory, and stored in an amber jar in the

[1]Lederle Products Department, Cyanamid of Canada Ltd., Montreal, Quebec

refrigerator at home. A 10 ml aliquot of this prepared folic acid solution was distributed in amber jars each week.

Supplements were calculated to provide 100 ug of total folate per day as 300 ml of reconstituted orange juice, or as one ml of prepared folic acid solution. The folic acid supplement was measured with a disposable plastic syringe, and was consumed with 120 ml of tap water. Half of each supplement was taken in the morning and in the evening.

C. Calculation of Nutrient Content of Folate-restricted Diet

1. Collection of dietary data

a. Food intake records

Each subject kept a food intake record during each of the fifth and ninth weeks of the folate-restricted diet, coinciding with the period when blood samples were taken. Data were collected for seven consecutive day periods, including a weekend. Subjects received directions for measuring and weighing food, and reporting recipes prior to the commencement of the dietary surveys.

Twenty-four subjects measured the quantity of food eaten. The measured food intake records served as a check on diet compliance, and were not used further for any calculations. The other 36 subjects were dietitians and dietetic interns who were familiar with the weighed dietary survey technique. They weighed the quantity of food consumed during the seven-day periods to provide additional data about diets taken. All food intakes, either in the measured or weighed dietary surveys, were of edible portions of foods as eaten. Edible portion, as defined by Adams (1975), "refers to the part of a food item that is potentially edible and customarily eaten even though the product may require cooking or other preparation to render it edible". This term applies to such foods as bread, milk and boneless meat, which are totally edible, and to fruits, vegetables and other foods for which inedible parts have been removed before the food is weighed.

Each subject was given a lined booklet at the start of each of the two dietary surveys. All 60 subjects listed quantities ingested at each meal and snack period, estimated in standard household measures and/or grams; types and brand names of all foods eaten; and methods of food preparation. They also provided family recipes for prepared items and mixed dishes. Quantities of food items consumed by the 24 subjects keeping measured food intake records were recorded by weight (ounces), volume (cups, tablespoons, teaspoons) or portion size (slices), depending on the food item. In some instances, especially when dining out, the serving size was estimated.

Five hundred gram food scales were given to each of the 36 subjects keeping weighed food intake records. They were also provided with a manual, prepared for this purpose, containing a computer printout of the (United States Department of Agriculture) USDA-numbered foods and their corresponding nutrient composition. The initial nutrient data bank was based on the USDA Agriculture Handbook No. 8 (Watt and Merrill, 1963). Additional nutrient values for a limited number of fabricated or prepared brand-name foods not listed in Agriculture Handbook No. 8 were added to the data base. Using this manual, the subjects selected the USDA food number which best described the food portion. One page from this manual is illustrated in Appendix Table Avi. This USDA number and the corresponding weight for the food eaten was then recorded in the booklet at each meal.

b. Evaluation of food intake records

All food intake records were then collected one day after the termination of each of the dietary surveys. The author reviewed the weighed intake records for ambiguities, and filled in any omissions after speaking with the participants. Food models and various sized glasses and spoons were used as aids in judging portion sizes. The measured food intake records were checked for diet compliance. Two were so detailed in descriptions of food and weights, that the measures were converted to grams using USDA Agriculture Handbook No. 456 (Adams, 1975). In total, 36 weighed food intake records were computerized on each of the fifth and ninth weeks of study.

Individual forms were used for collecting information on the dietary intake of each subject. The author verified, coded and recorded the information. Records were coded according to a three-digit computer number (001 to 060) and date of collection. Each individual food item listed in the dietary records was assigned a food number from Agriculture Handbook No. 8 and a gram weight. Single foods which were not found in Agriculture Handbook No. 8 were assigned a number of another food which described the unlisted food as specifically as possible. Some of the prepared foods and mixed dishes not listed in Agriculture Handbook No. 8 were broken down to their constituent ingredients according to the family recipe. Each recipe ingredient was coded with the appropriate food number and the estimated gram weight consumed by the subject.

2. Preparation of data base for total food folate content

A data base of total food folate content was compiled from selected published tables, microbiological analysis of specific foods and calculation of standardized recipes for home-prepared foods. In total, 350 total folate values expressed on a fresh weight basis were added to the preexisting USDA Agriculture Handbook No. 8 nutrient data tape, and later used for computer analysis of the weighed dietary records.

a. Use of published tables

Information on the total folate content of foods was obtained by direct contact with food companies, and from published sources (Herbert, 1963; Streiff, 1971; Butterfield and Calloway, 1972; Hoppner et al., 1972; Posati and Orr, 1976; Hoppner et al., 1977; Perloff and Butrum, 1977). When references for a particular food were found in both Perloff and Butrum (1977) and Hoppner et al. (1972, 1977), the latter values for Canadian foods were chosen.

Data on folate retention in cooked foods are highly variable and were not used. Only folate data obtained by the Lactobacillus casei method, using ascorbic acid protection and conjugase to free the bound forms, were chosen from the literature. All total folate values for individual foods were matched to the corresponding USDA food number, and were expressed per 100 g of fresh weight of the edible portion. The term "fresh" was used to describe foods that were purchased without later being processed (i.e. cooked).

b. Assay of foods from diet

Folate assay of foods without previously reported values was carried out at the end of the clinical trial. Forty food items were purchased from retail outlets in Montreal during the study period for this purpose. As many brands (median 4) as possible of a single food item were obtained, ranging from one for some soups and cereals to 22 for commercial rolls. A 100-200 g representative sample of the foods as purchased was prepared, collected in 240 ml polystyrene plastic containers and processed immediately. Moisture content of the fresh samples was determined on a small portion using the vacuum oven method (Horwitz et al., 1975). The remainder of the sample was lyophilized at -40°C, ground immediately after in a mortar and stored at -20°C until assayed. Moisture content was redetermined prior to microbiological analysis. The dried ground food was assayed for total folate content as described below.

For analysis seven ml of 0.05 M phosphate buffer (pH 8.0) containing 0.2% ascorbic acid was added to 0.5 g freeze-dried food sample in a 15 ml graduated centrifuge tube. These food extracts were thoroughly mixed, autoclaved in a steam bath (100°C) for 10 minutes and cooled in cold water. All extracts were assayed for total folate content with Lactobacillus casei ATCC 7469 by an adaptation (Hoppner et al., 1972) of the methods recommended by Herbert and Bertino (1967) and Baker and Frank (1967).

To assay total folate, desiccated chicken pancreas[1] was suspended

[1]Difco Laboratories, Detroit, Michigan

in deionized water[1] (6 mg/ml), extracted for 20 minutes and centrifuged for 5 minutes. One ml of the supernatant from the extracted chicken pancreas enzyme solution was added to each tube containing seven ml of food extract. The contents were adjusted to 10 ml with phosphate-ascorbate buffer. The mixture was incubated at 37°C in a water bath for 18 hours. After further mixing, centrifugation and appropriate dilution, the food extracts were assayed. The microbiological growth was determined as described by Herbert and Bertino (1967) and Baker and Frank (1967). The turbidity was measured at 660 nm after incubation of the cultures at 37°C for 22 hours. Final results were corrected for the enzyme blanks.

Data were reported per 100 g of fresh weight of the edible portion. All freeze-dried food samples were analyzed in duplicate on two different occasions. All foods that were drained before measuring were described as "drained solids". Foods containing liquid that was not drained were described as "solids and liquids". When possible, terms used to describe the various forms of a food were the same as those given in Agriculture Handbook No. 8.

c. Calculation of total folate content of home-prepared foods

The total folate content of the home-prepared foods which were eaten while taking the folate-restricted diet was not available from published

[1]Distilled water passed through Barnstead Ion X-change and Organic Removal columns (Barnstead Still and Sterilizer Co., Boston, Massachusetts)

sources. Calculated values were thus necessary. For the analysis of the weighed dietary records, folate values for 92 home-prepared foods were computed using recipes in the supplement to Agriculture Handbook No. 8 (Merrill et al., 1966) and adjusted for changes in weight occurring during cooking. The loss of folate activity in cooking was not accounted for. This condition permitted expression of total folate on a fresh weight basis. The home-prepared foods for which total folate was calculated were milk and fruit puddings, breads and rolls, baked desserts (cakes, pies, cookies) and main-course dishes (stews, potpies, pizzas).

Mixed dishes and home-prepared foods which were eaten were coded according to Agriculture Handbook No. 8. Only the total folate was computed for these foods. For unlisted items, ingredients from the recipe used for preparation were coded as separate foods and the entire recipe analyzed for nutrient content. A complete nutritive profile was calculated for six recipes.

The procedure followed in calculating the nutritive values and/or total folate of home-prepared foods and mixed dishes is illustrated in Table 3. This table shows the calculation of total folate for a 100 g portion of each of cake and custard filling for Boston Cream Pie, item 522 in Agriculture Handbook No. 8. Table 4 illustrates the calculation of total folate for 100 g Boston Cream Pie with the cake, filling and icing combined. Similar procedures were used in calculating total folate for foods composed of separate portions of cake, crust, topping, filling and icing.

The methodology used in calculating the nutritive values and/or

Table 3 Sample calculation of total folate in a home-prepared food from ingredients

Description - Boston cream pie with custard filling and powdered sugar topping (USDA No. 522)

Ingredient and other data	Measure	USDA No.	Weight (g)	Water (g)	Total folate (ug) calculation	
					Manual	Computer
Recipe I (Recipe factor 0.14)						
Cake portion						
Eggs, whole, fresh	2 large	968	146	107.6	30.8	4.3[b]
Cooking fat	1/4 cup	999	47	-	-	-
Milk, whole, fluid	1/2 cup	1320	121	105.8	6.0	0.8
Sugar, granulated, white	1 cup	2230	196	1.0	-	-
Flour, cake	1 2/3 cup	2443	198	23.8	-	-
Baking powder	1 1/2 tsp.	-	6	-	-	-
Salt	1/4 tsp.	-	2	-	-	-
Vanilla	1 tsp.	-	5	-	-	-
Total			721	238.2	36.8	5.1
Losses in cooking[a]			72.1	72.1	3.68	
Cooked product						
Total cake			648.9	166.1	33.1	
100-gram portion			100	25.6	5.1	5.1
Recipe II (Recipe factor 0.13)						
Filling portion						
Eggs, whole, fresh	2 large	968	146	107.6	30.8	4.0
Milk, whole, fluid	2 cups	1320	482	421.3	24.1	3.1
Sugar, granulated, white	1/2 cup	2230	98	0.5	-	-
Flour, all-purpose	1/3 cup	2439	48	5.8	10.2	1.3
Salt	1/4 tsp.	-	2	-	-	-
Vanilla	1 tsp.	-	5	-	-	-
Total			781	535.2	65.1	8.4
Losses in cooking[a]			78.1	78.1	6.51	
Cooked product						
Total filling			702.9	457.1	58.6	
100-gram portion			100	65.0	8.4	8.4

[a]Loss of 10 percent applied for evaporation in cooking

[b]Recipe factor = 100/721 = 0.14; Total folate (ug) x recipe factor: 30.8 ug x 0.14 = 4.3 ug

Table 4 Proportion of total folate from cake and filling/icing in cooked product

Description - Boston cream pie with custard filling and powdered sugar topping (USDA No. 522)

Description	Proportion of cooked weight of product[a]	Total folate content (ug)	
		100 g of components[b]	100 g of combined product
Boston Cream Pie			
Cake portion (Recipe I)	71%	5.1	3.6
Custard filling (Recipe II)	27%	8.3	2.2
Sugar icing	2%	-	-
Total	100%		5.8

[a]From Merrill et al. (1966), pg. 17

[b]From sample calculation Table 3

total folate was as presented below. All 92 home-prepared foods were computer calculated.

(1) Assign USDA food numbers to the recipe ingredients. (2) Convert the measures of the ingredients to corresponding gram weights (adapted from the Handbook of Food Preparation (1971), Agriculture Handbook No. 456 (Adams, 1975) and Fulton et al. (1977). (3) Multiply the weights of the separate ingredients by their nutritive values per gram (derived from values on the USDA nutrient data tape). (4) Adjust the total weight and nutritive values of the combined ingredients for weight changes occurring during cooking. A different factor was used for each home-prepared food. Changes in weight represented a loss in weight by evaporation of water. Since limited data are available on folate composition of cooked foods, folate destruction during cooking was not considered. Instead, the folate content was adjusted for the change in weight only. The quantity of folate in food decreased because of the loss of weight in cooking. (5) Convert the net totals to the 100 g basis, permitting use of the USDA nutrient data tape.

When information on the change in weight that occurs in cooking was lacking for a prepared food but data were available on its moisture content, the loss by evaporation was estimated. The estimate was made from an equation based on the calculated total weight and total water content of the uncooked ingredients in the recipe in relation to the percentage of water noted in analyzed values for the cooked product. When information on both weight change during cooking and water content was lacking, the loss by evaporation was estimated from the loss found for a similar type of food.

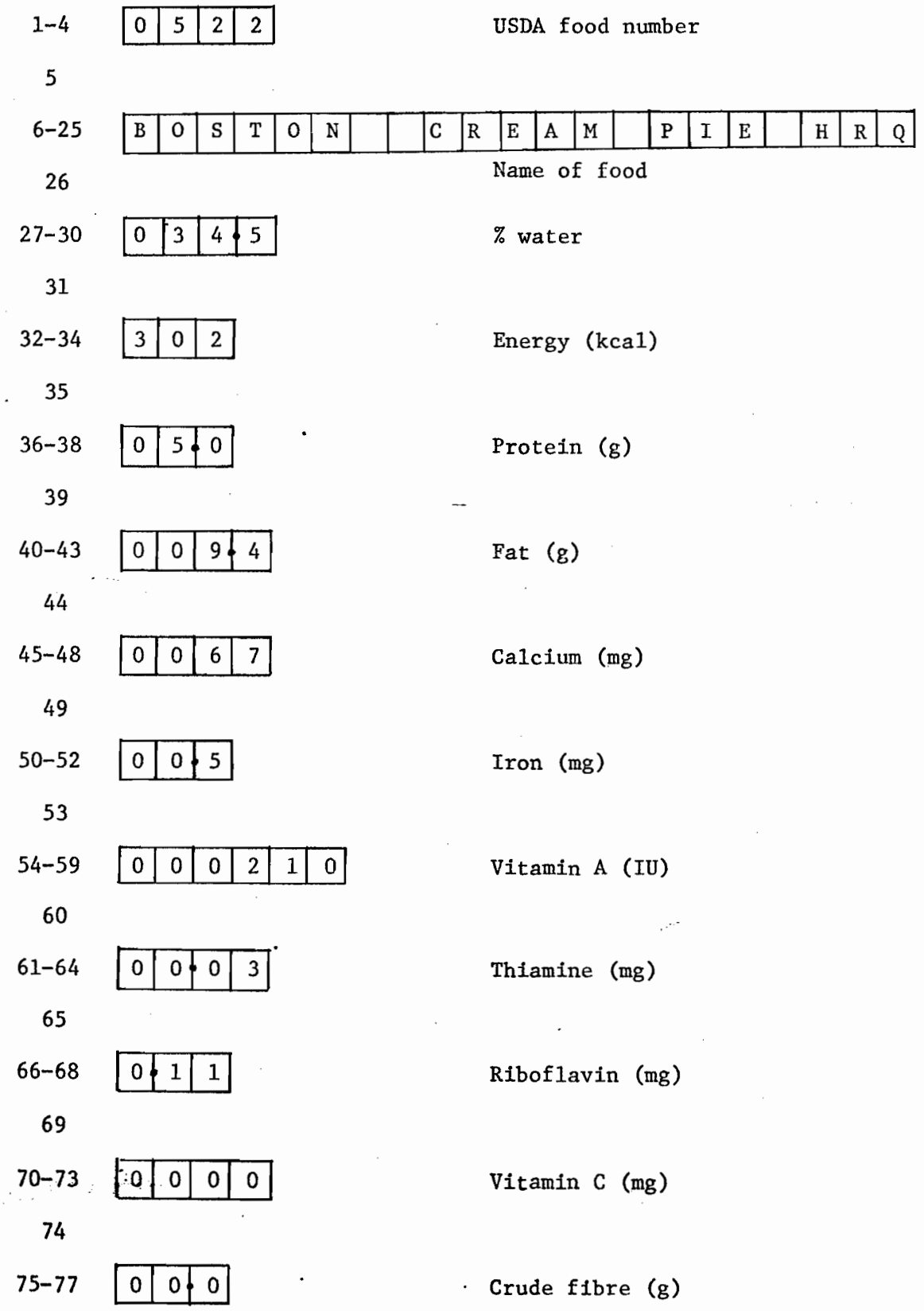
In the computer analysis of the recipes, the amount of the nutrient present in the gram weight of each ingredient was multiplied by a recipe factor. This factor was calculated for each recipe as 100/x, where x is the gram weight of the prepared food. The values obtained after multiplication by the factor were added together to give the total nutrient content of the recipe on a 100 g basis (Table 3). Following calculation of the 92 home-prepared foods by computer, the nutritive values and/or total folate not listed on the USDA nutrient data tape were added, as shown in Figures 2 and 3. These data were later used in the nutrient analysis of weighed dietary records, as described in the section on the analysis of dietary data by computer. The recipe information was fed into the computer as if it were the total intake of an individual being surveyed for one day.

3. Computer analysis of dietary data

a. Calculation using USDA nutrient data tape

Data compiled were keypunched onto cards using an IBM 029 card punch. Three card decks were prepared: (1) name cards, (2) food cards and (3) nutrient requirement cards. Each subject was assigned a three-digit computer number (000), which was punched onto an IBM card with the subject's name and days of the survey. This was the name card. Food cards listed the computer number, the four-digit USDA food number (0000) and the gram weight eaten as three figures (000). A new food card was punched for every food or recipe ingredient eaten. The nutrient requirement card listed the computer

Figure 2 Nutrient content of food items added to computer program (per 100g cooked weight)



From Watt and Merrill (1963), pg.19

Figure 3 Folic acid content of food items added to computer program (per 100g fresh weight)

Columns		
01-04	0 5 2 2	USDA food number
05		
06-25	B O S T O N C R E A M P I E H R Q	Name of food
26		
27-30	0 0 0.0	Free folate (ug)
31		
32-35	0 0 5.8	Total folate (ug)

From Merrill et al. (1966), pg. 13 and 16

number, subject's name, weight in pounds, age in years, activity level and sex.

All the food cards, plus the single name card and nutrient requirement card for each subject, were collected and fed into the IBM 3780 computer terminal at Macdonald College (McGill University). The data were processed by an IBM 370-115 computer system. A COBOL program (Farmer and Baker, 1981) and a nine-track tape, containing data from USDA Agriculture Handbook No. 8 and modified for our purposes, were used to analyze quantities of foods eaten. The computer, using the USDA nutrient data tape and the data cards, printed for each subject the food number, food name, amount consumed in grams and the nutrient content of each of the foods which were eaten. Each week of dietary survey was analyzed separately. Two computer outputs were obtained for each subject, and were made available to the participants. One printout is shown in Table 5.

Weekly nutrient intake was converted to daily intake by dividing by seven. Total and mean daily intakes of energy (kcal), protein (g), fat (g), calcium (mg), iron (mg), vitamin A (IU), thiamine (mg), riboflavin (mg), vitamin C (mg) and total folate (ug) were calculated for each subject. Nutrient intake and statistical analyses were computed with mean figures. Total folate intake was analyzed independent of the nine other nutrients. An illustration of the mathematical calculation performed by the computer is shown below.

Table 5 Sample computer printout of nutrient intakes for one subject listed by individual foods

NO. 1 NAME DAYS 7

DIET SURVEY

FOOD ITEM		TOTAL WT. G	PCT WATER	ENERGY CALORIES	PROTEIN GRAMS	FAT GRAMS	CALCIUM MG	IRON MG	VIT A I.U.	THIAM MG	RIBO MG	VIT C MG
13	APPLE R +SKIN	199	84.4	115.4	0.40	1.19	13.9	0.60	179.1	0.06	0.04	8.0
27	APPLE JC CN/BOTTLE	439	87.8	206.3	0.44	0.00	26.3	2.63	0.0	0.04	0.09	4.4
141	BANANA R COMMON	448	75.7	380.8	4.93	0.90	35.8	3.14	851.2	0.22	0.27	44.8
353	BEEF ROUND STEAK C D	90	54.7	234.9	25.74	13.86	10.8	3.15	27.0	0.07	0.20	0.0
370	BEEF HAMBURGER L	190	54.2	543.4	45.98	38.57	20.9	6.08	76.0	0.17	0.40	0.0
401	WINE TABLE BEV	61	85.6	51.9	0.06	0.00	5.5	0.24	0.0	0.00	0.01	0.0
473	BREAD WW H2O	535	36.4	1289.4	48.69	13.91	449.4	12.31	0.0	1.61	0.54	0.0
474	BREAD WW H2O T	101	24.3	289.9	10.91	3.13	101.0	2.73	0.0	0.29	0.12	0.0
505	BUTTER SALTED	26	15.5	186.2	0.16	21.06	5.2	0.00	858.0	0.00	0.00	0.0
522	BOSTON CREAM PIE HRQ	130	34.5	392.6	6.50	12.22	87.1	0.65	273.0	0.04	0.14	0.0
526	Q CHOC+CHOC ICING HR	280	22.0	1033.2	12.60	45.92	196.0	2.80	448.0	0.06	0.23	0.0
534	PLAIN CUPCAKE NO ICE	30	24.5	109.2	1.35	4.17	19.2	0.12	51.0	0.01	0.03	0.0
541	WHITE CAKE NO ICING	210	24.2	787.5	9.66	33.60	132.3	0.42	63.0	0.02	0.17	0.0
586	CHOC SWEET CANDY	36	0.9	190.1	1.58	12.64	33.8	0.50	3.6	0.01	0.05	0.0
649	CHEESE CREAM	42	51.0	157.1	3.36	15.83	26.0	0.08	646.8	0.01	0.10	0.0
651	CHEESE PARMESAN	30	30.0	117.9	10.80	7.80	342.0	0.12	318.0	0.01	0.22	0.0
653	CHEESE PROC AMER	60	40.0	222.0	13.92	18.00	418.2	0.54	732.0	0.01	0.25	0.0
682	CHICK ROAST L MEAT	170	63.8	282.2	53.72	5.78	18.7	2.21	102.0	0.07	0.17	0.0
684	CHICK ROAST DARK	205	64.4	360.8	57.40	12.92	26.7	3.49	307.5	0.14	0.47	0.0
800	COFFEE DR PD H2O BEV	960	98.1	9.6	0.00	0.00	19.2	0.96	0.0	0.00	0.00	0.0
812	COOKIE ASSRT PKG COM	40	2.6	192.0	2.04	8.08	14.8	0.28	32.0	0.01	0.02	0.0
818	COOKIE CHOC CHIP COM	120	2.7	565.2	6.48	25.20	46.8	2.16	144.0	0.05	0.08	0.0
848	CORN CN KERNEL VAC#Y	60	75.5	49.8	1.50	0.30	1.8	0.30	210.0	0.02	0.04	3.0
924	CRANBERRY SC HR SW	89	53.9	158.4	0.18	0.27	6.2	0.18	17.8	0.01	0.01	1.8
929	CREAM LIGHT COFFEE	30	71.5	63.3	0.90	6.18	30.6	0.00	252.0	0.01	0.05	0.3
943	CUCUMBER R PARED	50	95.7	7.0	0.30	0.05	8.5	0.15	0.0	0.02	0.02	5.5
974	EGGS CHICK HARDCOOK	100	73.7	163.0	12.90	11.50	54.0	2.30	1180.0	0.09	0.28	0.0
010	FISHCAKE C FRIED	90	66.0	154.8	13.23	7.20	0.0	0.00	0.0	0.00	0.00	0.0
032	GELATIN DESSERT H2O	120	84.2	70.8	1.80	0.00	0.0	0.00	0.0	0.00	0.00	0.0
141	ICE CREAM 16% FAT	64	62.8	142.1	1.66	10.30	49.9	0.00	422.4	0.01	0.07	0.6
259	LETTUCE R LOOSE LEAF	184	94.0	33.1	2.39	0.55	125.1	2.58	3496.0	0.09	0.15	33.1
300	MACARONI ENR SOFT C	70	72.0	77.7	2.38	0.28	5.6	0.63	0.0	0.10	0.06	0.0
317	MARGARINE	10	15.5	72.0	0.06	8.10	2.0	0.00	330.0	0.00	0.00	0.0
320	MILK WHOLE 3.5%FAT	144	87.4	93.6	5.04	5.04	169.9	0.00	201.6	0.04	0.24	1.4
322	MILK SKIM	328	90.5	118.1	11.81	0.33	396.9	0.00	0.0	0.13	0.59	3.3
323	MILK 2%FAT	1550	87.0	914.5	65.10	31.00	2216.5	1.55	1240.0	0.62	3.26	15.5
354	MUSHROOM COM RAW	55	90.4	15.4	1.49	0.17	3.3	0.44	0.0	0.06	0.25	1.7
499	PEANUT BUTTER FAT SG	45	1.7	265.1	11.34	22.77	26.6	0.86	0.0	0.05	0.05	0.0
524	PEAS GN CN SW DRAIN	55	79.0	44.0	2.53	0.22	13.8	0.94	379.5	0.06	0.03	4.4
611	PINEAPPLE RAW	80	85.3	41.6	0.32	0.16	13.6	0.40	56.0	0.07	0.02	13.6
770	PORK HAM CR LEAN R	30	66.4	50.4	6.45	2.55	3.6	0.96	0.0	0.27	0.07	0.0
789	POTATOES FRENCH FRY	83	44.7	227.4	3.57	10.96	12.5	1.08	0.0	0.11	0.07	17.4
793	POTATO MASH+MILK+FAT	300	79.8	282.0	6.30	12.90	72.0	1.20	510.0	0.24	0.15	27.0
873	RICE UNER C	70	72.6	76.3	1.40	0.63	7.0	0.14	0.0	0.01	0.01	0.0
891	RICE PUDDING RAISINS	180	65.8	262.8	6.48	5.58	176.4	0.72	198.0	0.05	0.25	0.0
899	ROLL COM DANISH PSTY	240	22.0	1012.8	17.76	56.40	120.0	2.16	744.0	0.17	0.36	0.0
902	ROLLS COM PLAIN ENR	160	31.4	476.8	13.12	8.96	118.4	3.04	0.0	0.45	0.29	0.0

continued

Table 5 (continued)

NO. 1 NAME DAYS 7

DIET SURVEY

FOOD ITEM		TOTAL WT. G	PCT WATER	ENERGY CALORIES	PROTEIN GRAMS	FAT GRAMS	CALCIUM MG	IRON MG	VIT A I.U.	THIAM MG	RIBO MG	VIT C MG
1932	FRENCH DRESSING	12	38.8	49.2	0.07	4.67	1.3	0.05	0.0	0.00	0.00	0.0
1938	MAYONNAISE	37	15.1	265.7	0.41	29.56	6.7	0.19	103.6	0.01	0.01	0.0
2089	CRM MUSHRM SOUP DIL	120	89.6	67.2	1.20	4.80	20.4	0.24	36.0	0.01	0.06	0.0
2158	SPAGETTI C ENR PLAIN	120	63.6	177.6	6.00	0.60	13.2	1.32	0.0	0.22	0.12	0.0
2165	SPAGHET+TOM+MEAT HR	315	70.0	422.1	23.63	14.81	157.5	4.73	2016.0	0.32	0.38	28.4
2230	SUGAR GRANULATED WH	12	0.5	46.2	0.00	0.00	0.0	0.01	0.0	0.00	0.00	0.0
2282	TOMATOES RIPE RAW	50	93.5	11.0	0.55	0.10	6.5	0.25	450.0	0.03	0.02	11.5
2286	TOMATO CATSUP	30	68.6	31.8	0.60	0.12	6.6	0.24	420.0	0.03	0.02	4.5
2350	TURKEY POTPIE HR	165	56.2	391.1	17.16	22.28	44.6	2.31	2194.5	0.18	0.21	3.3
2460	WHEAT SHREDDED	52	6.6	184.1	5.15	1.04	22.4	1.82	0.0	0.11	0.06	0.0
2505	BROWN GRAVY	6	75.0	7.9	0.12	0.76	0.2	0.04	0.1	0.00	0.00	0.0
2543	RITZ	6	2.3	1.7	0.44	1.58	1.3	0.09	0.0	0.00	0.00	0.0
2761	SM YOGURT FRUIT FLVD	113	89.0	92.7	5.09	0.00	149.2	0.11	6.8	0.05	0.29	2.3
TOTAL				14338.3	567.12	577.48	6113.7	76.21	19576.5	6.53	11.13	235.7
AVERAGE				2048.3	81.02	82.50	873.4	10.89	2796.6	0.93	1.59	33.7

RECOMMENDED DAILY NUTRIENT INTAKE

WEIGHT KG	HEIGHT CM	AGE YEARS	SEX	ENERGY CALORIES	PROTEIN GRAMS	CALCIUM MG	IRON MG	VIT A UG RE	THIAM MG	RIBO MG	VIT C MG
56.0	161	22	F	2100.0	41.00	700.0	14.00	800.0	1.10	1.30	30.0

REFERENCE - HEALTH AND WELFARE CANADA. COMMITTEE FOR REVISION OF THE CANADIAN DIETARY STANDARD, BUREAU OF NUTRITIONAL SCIENCES. (REVISED 1974)

Wheat flours, all-purpose, enriched

Components	USDA nutrient data tape	Computer calculation
	Edible portion	
	100 g	6 g
Water (%)	12	12.0
Food energy (kcal)	364	21.8

Calculation of food energy (kcal) = 6 g x 364 kcal/100 g = 21.8 kcal

The adequacy of nutrient intake was evaluated by comparing the data with the 1975 Recommended Daily Nutrient Intake (Health and Welfare Canada, 1975) by age and sex. This was listed beneath each subject's dietary survey computer printout (Table 5). It included recommended intake levels for energy, protein, calcium, iron, vitamin A, thiamine, riboflavin and vitamin C. No standard exists for fat. All were shown in the same units as the survey values, except for vitamin A which appeared as ug RE (Retinol Equivalents). (Conversion equation vitamin A IU/3.3 = vitamin A ug RE).

b. Expression of total folate on fresh weight basis

The destruction of folate activity during cooking was not considered in the calculation of the total folate content of either individual foods or the weighed dietary intakes. Total folate values from the literature, microbiological analysis and recipe calculation were expressed on a fresh weight basis. The recorded total folate content of cooked foods was that contained in the weight of the raw or fresh food before cooking. Foods

for which cooked weights were recalculated to the uncooked weights were vegetables, legumes, dry cereal products, toasted bread, eggs, lean fish, poultry, meat and soups.

Changes in weight during cooking represent either a loss in weight, by evaporation of water (toasted bread), or an increase due to absorption of water (pasta, rice). Meat undergoes losses through evaporation of moisture and loss of fat in the drippings. Table 6 shows, for several retail cuts, the percent weight of cooked meat and poultry as compared to the raw form (Pecot and Watt, 1970; Adams and Richardson, 1977).

For data processing, IBM cards were punched for each food, with its corresponding raw and cooked USDA food numbers (i.e. 2157-2158 raw-cooked spaghetti). Meat and poultry were treated separately. These cards were run concurrently with the food cards (USDA food number and amount eaten) for each subject. A COBOL program was developed to compute the equivalent raw (fresh) weight from the cooked weight on the basis of dry weight as follows:

(1) Subtract % water from 100 for % solids content (dry weight). (2) Calculate conversion factor by dividing % solids of cooked weight by % solids of raw weight. (3) Multiply cooked weight by conversion factor, and (4) Multiply raw weight by ug total folate per 100 g fresh weight; divide by 100.

Table 6 Effect of preparation on weight of meat[a]

Description of food before preparation	Weight after preparation (less drippings)	
	Description	Percent of uncooked
Chops or steaks for broiling or frying		
Pork, lamb chops; sirloin steaks	Lean and fat	53
	Lean only	38
Veal rump or round; beef round	Lean and fat	78
	Lean only	66
Ground meat - beef, lamb, pork	Patties	69
Roast for oven cooking (no liquid added)		
With bone - cured ham, pork loin lamb lean, ham lean	Lean and fat	56
	Lean only	47
Without bone	Lean and fat	69
	Lean only	53
Cuts for pot roasting, simmering, stewing		
Beef chuck, pork shoulder	Lean and fat	53
	Lean only	44
Trimmed beef, veal chuck	Lean	63
Roasting poultry		
Chicken leg	Lean and fat	73
Chicken breast	Lean and fat	66
Turkey thigh	Lean and fat	81
Turkey breast	Lean and fat	89
Miscellaneous		
Fried chicken	Lean and fat	79
Bacon	Lean and fat	32
Frankfurters	Lean and fat	98
Pork sausage links	Lean and fat	45

[a]Adapted from Pecot and Watt (1970) and Adams and Richardson (1977)

Example 1

USDA Number	Food	Water (%)	Solids (%)	Weight (g)
2158	Spaghetti cooked	63.6	36.4	120
2157	Spaghetti raw	10.4	89.6	unknown

Factor: cooked solids/raw solids = 36.4/89.6 = 0.41

Cooked weight x factor = raw weight

120 g x 0.41 = 49 g

Total folate = 49 g x 13.2 ug/100 g (raw) = 6.4 ug (raw)

To obtain the raw weight of meat and poultry, the cooked weight was divided by the percent weight after cooking for the particular retail cut (Table 6). This calculation was performed by the author for each weight of cooked meat eaten. A new IBM food card was punched containing the recalculated raw weight of the meat. The computer then determined the total folate content in the raw meat. The following example illustrates the computation of the raw weight of meat.

Example 2

USDA Number	Food	Weight (g)
370	Beef hamburger cooked	190
369	Beef hamburger raw	unknown

Cooked weight/percent weight after cooking = raw weight

190 g/69% (Table 6) = 276 g

Total folate = 276 g x 7.7 ug/100 g (raw) = 21.2 ug (raw)

Table 7 Sample computer printout of total folate intake for one subject listed by individual foods

NO. 1 NAME DAYS 7
DIET SURVEY

FOOD ITEM		TOTAL WT. G	FIBRE GRAMS	FREE FOL GRAMS	TOT FOL GRAMS	FOLIC 3 GRAMS	CHOL. MG
13	APPLE R +SKIN	199	2.0	06.5	11.5	00.0	0
27	APPLE JC CN/BOTTLE	439	0.4	04.3	08.7	00.0	0
141	BANANA R COMMON	448	2.2	51.9	91.3	00.0	0
352	BEEF ROUND STEAK R D	115	0.0	05.0	08.2	00.0	0
369	BEEF HAMBURGER R	276	0.0	07.1	21.2	00.0	0
401	WINE TABLE BEV	61	0.0	00.0	00.0	00.0	0
473	BREAD WW H2O	655	9.8	112.6	356.3	00.0	0
505	BUTTER SALTED	26	0.0	00.0	00.7	00.0	65
522	BOSTON CREAM PIE HRQ	130	0.0	00.0	07.1	00.0	0
523	QCARAMEL NO ICING HR	30	0.0	00.0	01.5	00.0	0
526	Q CHOC+CHOC ICING HR	280	0.8	12.0	17.9	00.0	120
541	WHITE CAKE NO ICING	210	0.2	06.5	15.3	00.0	0
586	CHOC SWEET CANDY	36	0.2	00.0	00.0	00.0	0
649	CHEESE CREAM	42	0.0	00.0	05.5	00.0	47
651	CHEESE PARMESAN	30	0.0	00.0	02.1	00.0	34
653	CHEESE PROC AMER	60	0.0	00.0	04.8	00.0	54
681	CHICK LIGHT MEAT R	258	0.0	07.7	15.4	00.0	0
683	CHICK DARK MEAT R	281	0.0	14.0	30.9	00.0	0
800	COFFEE DR PD H2O BEV	960	0.0	00.0	00.0	00.0	0
812	COOKIE ASSRT PKG COM	40	0.0	00.0	11.7	00.0	0
818	COOKIE CHOC CHIP COM	120	0.5	05.4	11.2	00.0	0
848	CORN CN KERNEL VAC#Y	60	0.5	00.0	12.6	00.0	0
924	CRANBERRY SC HR SW	89	0.6	00.0	00.5	00.0	0
929	CREAM LIGHT COFFEE	30	0.0	00.4	00.6	00.0	20
943	CUCUMBER R PARED	50	0.2	06.0	07.5	00.0	0
968	EGGS CHICK R F&FR	100	0.0	11.9	21.1	00.0	504
1010	FISHCAKE C FRIED	90	0.0	00.0	20.7	00.0	0
1032	GELATIN DESSERT H2O	120	0.0	00.0	00.0	00.0	0
1141	ICE CREAM 16% FAT	64	0.0	00.0	01.2	00.0	42
1259	LETTUCE R LOOSE LEAF	184	1.3	44.3	43.6	00.0	0
1298	MACARONI ENR DRY	22	0.1	00.9	02.3	00.0	0
1317	MARGARINE	10	0.0	00.2	00.2	00.0	0
1320	MILK WHOLE 3.5%FAT	144	0.0	00.0	07.2	00.0	20
1322	MILK SKIM	328	0.0	00.0	16.4	00.0	7
1323	MILK 2%FAT	1550	0.0	00.0	93.0	00.0	140
1354	MUSHROOM COM RAW	55	0.4	11.1	12.7	00.0	0
1499	PEANUT BUTTER FAT SG	45	0.8	08.9	35.5	00.0	0
1524	PEAS GN CN SW DRAIN	55	1.2	00.0	11.3	00.0	0
1611	PINEAPPLE RAW	80	0.3	07.3	08.4	00.0	0
1770	PORK HAN CR LEAN R	30	0.0	00.0	03.3	00.0	0
1785	POTATOES RAW	527	2.6	52.1	66.9	00.0	0
1877	RICE BNEK RAW	22	0.1	03.3	06.3	00.0	0
1891	RICE PUDDING RAISINS	180	0.2	00.0	10.9	00.0	20
1899	ROLL COM DANISH PSTY	240	0.2	00.0	65.9	00.0	0
1902	ROLLS COM PLAIN ENR	160	0.3	13.1	56.6	00.0	0
1932	FRENCH DRESSING	12	0.0	00.0	00.0	00.0	0
1938	MAYONNAISE	37	0.0	00.1	00.3	00.0	26

continued

Table 7 (continued)

NO. 1 NAME DAYS 7

DIET SURVEY

FOOD ITEM		TOTAL WT. G	FIBRE GRAMS	FREE FOL GRAMS	TOT FOL GRAMS	FOLIC 3 GRAMS	CHOL. MG
2088	CRM MUSHRM SOUP COND	60	0.1	00.4	01.6	00.0	0
2157	SPAGHETTI DRY ENR	49	0.1	01.8	06.4	00.0	0
2165	SPAGHET+TOM+MEAT HR	315	0.9	00.0	23.0	00.0	95
2230	SUGAR GRANULATED WH	12	0.0	00.0	00.0	00.0	0
2282	TOMATOES RIPE RAW	90	0.3	02.0	02.8	00.0	0
2285	TOMATO CATSUP	30	0.2	03.0	04.5	00.0	0
2350	TURKEY POTPIE HR	165	0.7	00.0	18.9	00.0	51
2460	WHEAT SHREDDED	52	1.2	04.9	26.1	00.0	0
2505	BROWN GRAVY	6	0.0	00.0	00.0	00.0	0
2543	RITZ	6	0.0	00.0	00.0	00.0	0
2761	SM YOGURT FRUIT FLVD	113	0.0	00.0	10.1	00.0	8
TOTAL			28.6	406.0	1241.7	00.0	1251
AVERAGE			4.1	58.0	177.4	00.0	179

A computer printout of the total folate intake for the subject whose dietary survey was presented in the above section is shown in Table 7.

c. Contribution of food groups to total folate intake

Nine major food groups used in the Nutrition Canada Food Consumption Patterns Report (1977) formed the basis for the food grouping system. Particular attention was focused on the vegetable group, which was further subdivided into the food categories of potatoes, leafy vegetables, root vegetables and garden fruits. The cereals were represented by two food groups: cereal products and breakfast cereals. The foods eaten by the 36 subjects were thus classified according to the 14 food groups presented in Table 8.

All food items in Agriculture Handbook No. 8 which were eaten were coded by a food group number (1 to 14). The individual dietary records were then computer analyzed. A specially designed COBOL program classified all the USDA-coded foods eaten by each subject according to the 14 food groups, and calculated the intakes for each nutrient by food groups. A computer printout of the total folate intake by food groups is illustrated for the same subject as mentioned above (Table 9). The total folate intake was calculated on the fresh weights of foods.

Table 8 Food group classification

Group Number	Food group	Food category	Food classification
1	Dairy products	Milk & milk products Cheese and cheese products	
2	Meat, poultry, fish	Meat, poultry, fish	
3	Eggs	Eggs	
4	Cereal products	Bread and rolls Pasta Grains and flour mixtures	
5	Breakfast cereals	Breakfast cereals	
6	Potatoes	Potatoes (except sweet)	
7	Leafy vegetables	Leafy vegetables	Cabbage, celery, lettuce
8	Nuts and legumes	Nuts and legumes	Dried peas, beans, nuts, seeds
9	Root vegetables	Root vegetables	Carrots, onion, turnips, sweet potatoes
10	Garden fruits	Garden fruits	Tomatoes, cucumbers, squash
11	Fruit and fruit products	Fruit and fruit products	Fresh, canned, frozen, dried, juices, nectars
12	Cooking oils and fats	Butter, margarine, oils, fats	
13	Sugar and adjuncts	Foods primarily sugar Beverages	Sugars, condiments Tea, coffee, soft drinks
14	Mixed dishes and soups	Mixed dishes Soups	Mixtures of food groups Soups

Table 9 Sample computer printout of total folate intake for one subject listed by food groups

NO. 1 NAME DAYS 7

DIET SURVEY

FOOD ITEM	TOTAL WT. G	FIBRE GRAMS	FREE FOL GRAMS	TOT FOL GRAMS	FOLIC 3 GRAMS	CHOL. MG
DAIRY PRODUCTS	2361	0.0	00.4	141.0	00.0	370
MEAT, POULTY, FISH	1050	0.0	34.0	100.0	00.0	0
EGGS	100	0.0	11.9	21.1	00.0	504
CEREAL PRODUCTS	2024	12.8	155.9	591.7	00.0	120
BREAKFAST CEREALS	52	1.2	04.9	26.1	00.0	0
POTATOES	527	2.6	52.1	66.9	00.0	0
LEAFY VEGETABLES	184	1.3	44.3	43.6	00.0	0
NUTS & LEGUMES	55	1.2	00.0	11.3	00.0	0
ROOT VEGETABLES	0	0.0	00.0	00.0	00.0	0
GARDEN FRUITS	155	0.8	19.2	23.0	00.0	0
FRUIT & FRUIT PRODUCTS	1166	5.0	70.2	120.1	00.0	0
OILS & FATS	130	0.8	09.3	36.8	00.0	91
SUGAR & ADJUNCTS	1308	1.0	03.0	05.0	00.0	0
MIXED DISHES & SOUPS	726	1.9	00.4	54.6	00.0	165
TOTAL		28.6	406.0	1241.7	00.0	1251
AVERAGE		4.1	58.0	177.4	00.0	179

4. Statistical analysis

Fifty-seven of the sixty subjects completed the study. Three had blood samples drawn for analysis at weeks 0, 2 and 5 and each completed one seven-day food intake record. Both nutrient and clinical data were analyzed using the Statistical Analysis System (SAS) computer programs (Barr et al., 1979). Data were analyzed by least squares analyses for unbalanced data using the General Linear Model (GLM) procedure (Barr et al., 1979). A completely randomized split-plot design was chosen for statistical testing. Using analysis of variance and the Student's t test, differences among the groups were considered significant when probability (P) was less than or equal to 0.05. If a difference was not significantly different, the designation NS was used.

Mean $\pm$ standard error of the mean (SEM) was calculated for all groups of data. Means were ranked by Duncan's multiple range test (Steel and Torrie, 1960). Grouping by Duncan's multiple range test was set at 5% significance level, with the magnitude of data decreasing in alphabetic order. Any two means in the same group were not significantly different from each other. The Student's t test was used only when two groups were involved in comparison. Statistical analyses for nutrient and clinical data are presented in Appendix B.

a. Nutrient data

The intakes for each nutrient from each of the fifth and ninth weeks were pooled for subsequent analyses. Nutrient intakes among groups were analyzed by multivariate analysis of variance. Mean values were used to express the nutrient data. Medians were included for comparison. Mean daily levels were compared to the 1975 Recommended Daily Nutrient Intake.

The vegetable, cereal and egg food groups were combined to give a total of nine food groups. The percent contribution of a food group to total intake was computed as the mean intake from the food group divided by the total mean intake from all food groups multiplied by 100. Food groups were defined as primary sources of total folate when they contributed the highest percentage of the intake to the diet and were secondary sources when they ranked second. Ethanol consumption from alcoholic drinks was calculated by multiplying the percent ethanol concentration by the quantity of alcoholic drinks consumed. Ethanol intakes between times were analyzed by unpaired Student's t test, and across time with analysis of variance.

b. Clinical data

Analyses of variance with repeated measures were used to determine differences in serum and erythrocyte folates within and among groups (Winer, 1971). Clinical data were also analyzed at each time period by analysis of variance and between times by paired and unpaired Student's t test.

Rserum, the ratio of serum folate at a sampling period to that at week 2, and RRBC, the similar ratio for erythrocyte folate, were calculated for all individual subjects at week 0 and week 9. Changes in serum and erythrocyte folate in individual subjects could thus be followed. Recalculation of data as the ratios Rserum and RRBC did not alter the interpretation of the data presented.

IV. RESULTS

A. Total Folate Content of Folate-restricted Diet

1. Foods from diet assayed for total folate

Folate content of 40 foods and food products eaten while on the folate-restricted diet was measured. Results are presented in Table 10. The total folate activity ranged from 7.9-32.3 ug/100 g for canned vegetables, 37.6-127.7 ug/100 g for frozen vegetables, 1.9-11.1 ug/100 g for canned fruit, 20.6-35.8 ug/100 g for cereal products and 3.9-19.0 ug/100 g for condensed soups. Differences in total folate content were observed for vegetables depending on the processing techniques and separation of solid and liquid portions. Higher total folate activity was reported for condensed soups containing a greater percentage of vegetables.

2. Folate content of restricted diet by food groups

Mean daily folate intake and contribution to this by food groups are shown in Table 11. Data are based on edible portions of the fresh weights of foods. The vegetable group, including potatoes, contributed the largest percentage (27%) of total folate, and cereal products were the second largest contributor (25%). The mean intake of vegetables was 191 g, composed of potatoes (47 g), leafy vegetables (52 g), root vegetables

Table 10 Total folate activity in foods measured with Lactobacillus casei

Food	USDA number	Number of samples	Moisture (%) Mean ± SD[a]	Total folate ug/100 g fresh weight Mean ± SD[a]
Vegetables, canned, solids and liquids				
Corn sweet cream style	0847	7	77.7 ± 0.2	32.3 ± 7.9
Corn sweet kernel vacuum	0848	8	74.7 ± 0.9	21.0 ± 8.3
Mushrooms commercial	1355	6	93.1 ± 0.1	7.9 ± 0.8
Tomato puree	2296	5	87.2 ± 0.3	17.2 ± 5.7
Vegetable juice cocktail	2396	3	94.5 ± 0.1	16.1 ± 0.1
Vegetables, canned, drained solids				
Beans snap green	0186	4	93.7 ± 0.3	19.6 ± 0.6
Beans snap yellow	0198	4	93.5 ± 0.5	26.1 ± 3.5
Beets common red	0387	4	90.1 ± 0.3	8.4 ± 3.2
Peas green sweet	1524	8	80.2 ± 0.1	20.7 ± 3.2
Vegetables, frozen, unthawed				
Beans snap green french style	0193	4	90.2 ± 0.5	37.6 ± 3.1
Broccoli spears	0487	3	90.0 ± 0.3	98.1 ± 6.6
Brussel sprouts	0491	3	83.0 ± 0.1	127.7 ± 9.0
Cauliflower	0632	2	93.4 ± 0.1	57.9 ± 2.3
Vegetables mixed	2403	4	82.0 ± 0.8	47.2 ± 2.8
Fruit, canned, syrup heavy, solids and liquids				
Fruit salad	1027	3	79.5 ± 0.3	7.2 ± 1.1
Peaches	1483	8	80.6 ± 0.2	11.1 ± 1.6
Pears	1507	4	80.1 ± 0.2	1.9 ± 0.1
Dairy, fish, luncheon meats				
Cheese process skim milk	-	3	55.1 ± 0.1	24.3 ± 0.5
Fish cakes cooked	1010	2	65.2 ± 0.8	23.1 ± 0.1
Fish sticks cooked frozen	1017	3	64.9 ± 0.4	8.0 ± 0.5
Sausage beef boneless smoked	-	4	66.7 ± 0.8	8.0 ± 0.4
Sausage salami cooked	2018	7	59.1 ± 0.7	20.7 ± 0.8
Cereals and cereal products				
Bran malt and sugar	0439	3	3.2 ± 0.1	35.1 ± 0.1
Bran flakes raisins thiamine	0442	1	9.1 ± 0.6	20.6 ± 7.7
Cookies assorted packaged	0812	20	3.5 ± 0.1	29.4 ± 3.7
Oat whole wheat cereal sweet	-	1	2.4 ± 0.1	22.8 ± 0.4
Rolls buns commercial	1899	22	27.4 ± 1.0	35.8 ± 11.6

continued

Table 10 (continued)

Food	USDA number	Number of samples	Moisture (%) Mean ± SD[a]	Total folate ug/100 g fresh weight Mean ± SD[a]
Miscellaneous				
Mixed dishes, cooked, frozen				
Egg rolls with meat	-	4	53.8 ± 1.0	23.2 ± 0.1
Tourtiere commercial	-	4	47.4 ± 0.2	17.6 ± 4.1
Soups, commercial, canned, condensed				
Beef vegetable barley	-	1	84.2 ± 0.1	14.5 ± 0.4
Cream celery	2068	2	90.1 ± 0.1	5.9 ± 0.1
Chicken consomme	2071	1	94.3 ± 1.1	3.9 ± 0.9
Cream chicken	2073	3	84.9 ± 0.1	5.6 ± 0.6
Chicken noodle	2078	8	87.8 ± 0.1	5.5 ± 0.1
Minestrone	2086	2	87.5 ± 0.1	8.6 ± 1.7
Onion	2091	1	86.9 ± 0.1	17.9 ± 4.3
Pea green	2093	1	70.3 ± 0.1	19.0 ± 0.7
Pea split	2096	2	85.2 ± 0.2	11.2 ± 2.0
Vegetarian vegetable	2107	4	86.2 ± 0.1	14.4 ± 0.1
Soups, dehydrated, dry form				
Chicken rice	2113	4	5.3 ± 0.2	55.8 ± 2.4

[a]Values are mean ± SD for replicates in assays

Table 11 Mean daily dietary folate intake and percent contribution by food groups

Food group	Intake/person/day		Percent contribution
	Consumption (g)	Total folate (ug)	
Dairy products	304	21	13
Meat, poultry, fish, eggs	165	16	10
Cereal products	159	40	25
Fruit and fruit products[a]	227	19	12
Vegetables and potatoes	191	41	27
Nuts and legumes	23	10	6
Cooking oils and fats	20	2	1
Sugars and adjuncts	346	1	1
Mixed dishes/soups	80	9	5
Total	1515	159	100

[a]Excludes orange juice supplementation

(25 g) and garden fruits (67 g). The percentage contribution of the individual categories to total folate intake were 4, 13, 4 and 6%, respectively. Mean consumption of cereal products was 148 g, including 11 g of breakfast cereals. Although an average of 227 g of fruit and fruit products were consumed daily, these contributed only 12% of total dietary folate.

B. Nutrient Intake During Folate-restricted Diet

The nutrient intakes of the 36 women in the total study who weighed their seven-day dietary intakes are described below. The 24 women who did not weigh their individual diets reported similar food consumption patterns.

1. Nutritional adequacy of the folate-restricted diet

Nutrient intakes for the 36 subjects are shown as percentages of the 1975 Recommended Daily Nutrient Intake (RDNI) (Table 12). RDNI table values for the reference woman, age 19-35 years, were used as the standard for comparison. Mean values exceeded two-thirds of the RDNI for energy and all nutrients. In all categories except energy, iron, thiamine and total folate, the mean intakes actually exceeded the RDNI. Mean protein, vitamin A and vitamin C intakes were considerably greater than the recommended allowances. Individual intakes were less than two-thirds of recommended for calcium in five (14%), and for riboflavin in four (11%) subjects. Three subjects reported minimal intakes of both nutrients. Excluding folate supplements, mean folate intake was below that recommended by RDNI, with 54% of the total group consuming at least two-thirds of the quantity recommended. Despite restriction in dietary folate intake, mean folate intake of these subjects was 80% of the RDNI. For all 36 women, 39% of energy intake was supplied by fat and 17% by protein.

Table 12 Mean daily nutrient intake of all 36 subjects compared to 1975 Recommended Daily Nutrient Intake (RDNI)

Nutrient	Intake[a] (n = 36)	RDNI (Women)[b]	% RDNI
Energy (kcal)	1699 ± 42	2100	81
Protein (g)	71 ± 2	41	173
Fat (g)	74 ± 3	70[e]	106
Calcium (mg)	811 ± 36	700	116
Iron (mg)	11.0 ± 0.2	14	79
Vitamin A (IU)[c]	5347 ± 250	2700	198
Thiamine (mg)	1.08 ± 0.04	1.10	98
Riboflavin (mg)	1.52 ± 0.05	1.30	117
Vitamin C (mg)	75 ± 4	30	250
Total folate (ug)	159 ± 5[d]	200	80[d]

[a]Mean ± SEM

[b]Reference woman is 19-35 years old, 56 kg (123 lbs), 161 cm (5'3")

[c]Reference: 1 RE = 3.33 IU; RE = Retinol Equivalent;
IU = International Units; RDNI = 800 ug RE

[d]Excludes folate content of orange juice and folic acid supplements

[e]No RDNI available. Fat allowance calculated as 30% of total calories

2. Comparison of nutrient intakes

The mean and median daily nutrient intakes of the 36 women during the folate-restricted diet are listed in Tables 13 and 14. Statistical analysis of the restricted diet revealed no significant difference among the three treatments in intake of energy, protein, fat, calcium, iron, thiamine, riboflavin and total folate. Vitamin A and C intakes were higher in the folic acid group than in the others ($P<0.05$) (Table 14).

The percentage of calories derived from fat was higher for the orange juice group (41%) than for either the control or folic acid (38%) groups. Protein contribution to energy intake was similar among all three treatments (17%) (Table 13). The energy derived from alcoholic drinks consumed outside of the two dietary survey weeks was excluded from all calculations of caloric intake.

The percentages of the RDNI for energy consumed were 82, 79 and 83%, respectively, for the control, orange juice and folic acid groups. Three subjects in the folic acid group and five in each of the control and orange juice groups consumed less than two-thirds of the RDNI for iron (less than 9.4 mg). Mean thiamine intake of the orange juice group was 94% of the RDNI, while the control and folic acid groups exceeded the allowance for thiamine.

Calculations of total folate intake excluded folate content of the orange juice and folic acid supplements. The mean folate intakes excluding supplements as a percentage of the allowance were 82, 76 and 85%, respectively, for the control, orange juice and folic acid groups. Sixteen

Table 13 Mean and median daily macronutrient and mineral intakes of nonsupplemented and folate supplemented subjects during folate-restricted diet

Folate supplement		Macronutrients			Minerals	
		Energy (kcal)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)
Nil (n = 11)	Mean	1715	69(17%)[a]	73(38%)[b]	824	11.0
	SEM	73	3	5	63	0.4
	Median	1675	73	68	841	11.2
Orange juice (n = 15)	Mean	1659	70(17%)[a]	75(41%)[b]	763	10.6
	SEM	66	3	5	49	0.4
	Median	1621	69	69	756	10.5
Folic acid (n = 10)	Mean	1743	74(17%)[a]	75(38%)[b]	863	11.1
	SEM	88	4	5	77	0.5
	Median	1741	75	68	744	10.5

[a] Percentage of energy derived from protein

[b] Percentage of energy derived from fat

Table 14 Mean and median daily vitamin intakes of nonsupplemented and folate supplemented subjects during folate-restricted diet

Folate supplement		Vitamins				
		Vitamin A (IU)	Thiamine (mg)	Riboflavin (mg)	Vitamin C (mg)	Total folate[a] (ug)
Nil (n = 11)	Mean	5286	1.10	1.53	74	163
	SEM	558	0.08	0.09	8	9
	Median	4973	1.05	1.60	67	154
Orange juice (n = 15)	Mean	4774	1.03	1.46	66	151
	SEM	361	0.05	0.07	7	9
	Median	4679	1.01	1.44	56	138
Folic acid (n = 10)	Mean	6242*	1.12	1.61	91*	169
	SEM	493	0.07	0.11	7	7
	Median	5749	1.09	1.48	96	158

[a]Excludes folate content of orange juice and folic acid supplements

*Significantly different from values in treatment groups at $P < 0.05$

women consumed less than two-thirds of the recommendation for folate (less than 134 ug), seven of whom were in the orange juice group. Average daily folate intake was less than 100 ug in four women, all of whom were receiving orange juice. Mean folate intakes were 75 and 82% of the RDNI, respectively, for OCA users and nonusers. Four OCA users and 12 nonusers consumed less than two-thirds of the recommended allowance for folate. Mean folate consumption of the OCA users was 149 $\pm$ 7 ug/day (median 139), and of the 28 nonusers was 163 $\pm$ 6 (median 159) ug/day. Mean folate content of the diets of the 36 subjects weighing their food was 159 $\pm$ 5 ug/day (median 150), and of the 19 women receiving no folate supplement was 163 $\pm$ 9 (median 154) ug/day.

3. Comparison of ethanol intakes

The mean ethanol consumption of subjects who recorded intakes was 5.9 $\pm$ 0.8 ml/day at week 5 to 9 and 9.8 $\pm$ 1.4 ml/day at week 9 to 11 (Table 15). No significant difference was observed in ethanol intake among treatment groups at either blood sampling intervals. Ethanol intake increased over the six-week period, with mean consumption being highest in the orange juice group. The control group appeared to consume less than the other two groups, but the difference was not statistically significant. Mean energy intake from ethanol was 41 kcal at week 5 to 9 and 69 kcal at week 9 to 11.

Table 15 Mean daily ethanol intake during last two blood sampling intervals (week 5 to 11)[a]

	Ethanol, ml		
	Blood sampling intervals		
Folate supplement	Week 5 to 9	Week 9 to 11	P[c]
Nil	3.5 ± 0.7 (8)[b]	5.3 ± 1.4 (15)	NS
Orange juice	6.3 ± 1.4 (14)	12.2 ± 2.8 (17)	NS
Folic acid	6.9 ± 1.5 (12)	11.2 ± 2.5 (17)	NS
P[d]	NS	NS	
Total group	5.9 ± 0.8	9.8 ± 1.4	

[a]Values represent Mean ± SEM

[b]Sample number

[c]Unpaired Student's t. test

[d]Analysis of variance, Duncan's multiple range test

C. Experimental Observations

1. Effect of initial two weeks of folate-restricted diet on blood folates in total group

Following two weeks of folate-restricted diet, serum and erythrocyte folates were significantly lower than at week 0 in the 60 women studied ($P<0.002$ and $P<0.0001$, respectively). This decrease in serum and erythrocyte folates was observed in both OCA users and nonusers (Table 16). Mean serum folate at the inception of the study was significantly lower in OCA users than nonusers ($P<0.01$), but erythrocyte folates were not different. The difference in serum folates between OCA users and nonusers decreased but remained significant after 2 weeks of the restricted diet ($P<0.05$). Erythrocyte folates also decreased during this period ($P<0.0001$), but the difference between OCA users and nonusers was not significant. Mean erythrocyte folate decreased by 42% in OCA users, and by 29% in nonusers.

2. Effect of continued restricted diet on blood folates in nonfolate supplemented control subjects

Serum folate did not change significantly during the seven weeks of folate-restricted diet (week 2 to 9), but erythrocyte folates continued to decrease (Table 17). From a mean of 311 at week 2, erythrocyte folate decreased to 244 ng/ml at week 9 ($P<0.005$). Mean serum folate remained outside the clinical limits of deficiency throughout the study. The

Table 16 Serum and erythrocyte folates in OCA users and nonusers before and after two weeks of restricted diet[a]

	Serum folate, ng/ml			Erythrocyte folate, ng/ml		
Group	Week 0	Week 2	P[b]	Week 0	Week 2	P[b]
OCA users (n = 21)	8.7 ± 0.8	7.2 ± 0.6	<0.005	481 ± 23	281 ± 14	<0.0001
Nonusers (n = 39)	16.5 ± 2.7	9.2 ± 0.6	<0.005	435 ± 21	308 ± 16	<0.0001
P[c]	<0.01	<0.05		NS	NS	
Total group	13.8 ± 1.8	8.5 ± 0.4	<0.002	451 ± 16	299 ± 12	<0.0001

[a]Values represent Mean ± SEM

[b]Paired Student's t test

[c]Unpaired Student's t test

Table 17 Serum and erythrocyte folates in nonfolate supplemented OCA users and nonusers following 7 weeks of restricted diet (week 2 to 9)[a]

	Serum folate, ng/ml			Erythrocyte folate, ng/ml		
Group	Week 2	Week 9	P[b]	Week 2	Week 9	P[b]
OCA users (n = 7)	6.3 ± 0.6	7.5 ± 0.6	NS	307 ± 14	230 ± 22	<0.01
Nonusers (n = 12)	8.4 ± 0.6	8.8 ± 0.6	NS	314 ± 31	253 ± 21	NS
P[c]	<0.05	NS		NS	NS	
Total group	7.6 ± 0.5	8.3 ± 0.5	NS	311 ± 20	244 ± 15	<0.005
Median	6.8	8.6		311	230	

[a]Values represent Mean ± SEM

[b]Paired Student's t test

[c]Unpaired Student's t test

reference values for nondeficiency of folate calculated in the assay laboratory were greater than 4 ng/ml for serum folate, and greater than 175 ng/ml for erythrocyte folate (Cooper and Lowenstein, 1964). Mean serum folate at week 9 was 8.3 $\pm$ 0.5 (median 8.6) ng/ml. None of the individual values of serum folate, and two of those for erythrocyte folate among these 19 nonfolate supplemented subjects were in the range of deficiency. The lowest serum folate was 4.7 ng/ml, and the lowest individual erythrocyte folate was 145 ng/ml. Mean serum folate of the OCA users at week 9 was 7.5 $\pm$ 0.6 (median 7.6) ng/ml, and of nonusers was 8.8 $\pm$ 0.6 (median 8.8) ng/ml. No difference in serum folate was observed between these two groups at week 9. Mean erythrocyte folates were not significantly different between OCA users and nonusers at week 9.

The change in serum and erythrocyte folates in each subject during the experimental period was examined by calculation of the ratios of individual serum and erythrocyte folates at week 9 compared to week 2 values. These serum and erythrocyte folate ratios were not statistically different between OCA users and nonusers in the nonfolate supplemented group. Values at week 9 for serum folates were 127 and 108% of week 2 values for OCA users and nonusers, respectively. Erythrocyte folates were 76 and 89% of week 2 values for OCA users and nonusers, respectively.

3. Availability of orange juice folate

As previously mentioned, serum folate decreased in the total group during the initial 2-week period of folate-restricted diet ($P<0.002$). Although the mean serum folate at the inception of the study was lower in the nonfolate supplemented controls than in either the orange juice or folic acid groups, this difference was not significant (Table 18). The decrease in serum folate between week 0 and week 2 was statistically significant in the control ($P<0.001$) and orange juice ($P<0.01$) groups, but not in the group designated to receive synthetic folic acid supplement. Serum folate did not change significantly during the subsequent seven weeks of observation in the control group, but increased ($P<0.05$) in the groups receiving orange juice or folic acid. Erythrocyte folate also decreased in the three treatment groups during the initial 2-week period ($P<0.0001$) (Table 19), and continued to decrease in the nonfolate supplemented controls during the subsequent seven weeks ($P<0.005$). This significant difference was initially observed at week 9. Erythrocyte folate in the orange juice and folic acid groups remained unchanged during the seven weeks of folate supplementation.

Although no difference was observed among treatment groups at week 2, serum and erythrocyte folates were significantly lower ($P<0.05$) in the control group at week 9 than in either folate-supplemented group. No difference was observed in serum or erythrocyte folates of women receiving supplements as orange juice or as folic acid over the seven-week supplementation period (Tables 18 and 19). Erythrocyte folates did not rise significantly during supplementation, and remained under the levels seen at the inception of the study. Serum folate in the control group and erythrocyte

Table 18 Effect of restricted diet and folate supplements on serum folate[a]

	Serum folate, ng/ml								
Folate supplement	Week 0	P[b]	Week 2	P[c]	Week 5	P[d]	Week 9[g]	P[e]	Week 11[g]
Nil (n = 19)	10.2 ± 0.8	<0.001	7.6 ± 0.5	NS	14.5 ± 4.4	NS	8.3 ± 0.4*	NS	9.6 ± 0.5*
Orange juice (n = 21)	14.2 ± 2.3	<0.01	9.4 ± 1.0	<0.05	16.7 ± 3.1	NS	14.5 ± 1.4	<0.05	17.0 ± 4.5
Folic acid (n = 20)	16.7 ± 4.9	NS	8.4 ± 0.7	<0.05	25.1 ± 5.3	NS	20.5 ± 5.8	<0.05	23.5 ± 5.9
P[f]	NS		NS		NS		<0.05		<0.05

[a] Values represent Mean ± SEM

[b] Paired Student's t test, week 0 to 2

[c] Paired Student's t test, week 2 to 5

[d] Unpaired Student's t test, week 5 to 9

[e] Unpaired Student's t test, week 2 to 9

[f] Analysis of variance, Duncan's multiple range test

[g] For each treatment group at week 9 and 11, n = 19

* Significantly different from values in treatment groups at P <0.05

Table 19 Effect of restricted diet and folate supplements on erythrocyte folate[a]

	Erythrocyte folate, ng/ml								
Folate supplement	Week 0	P[b]	Week 2	P[c]	Week 5	P[d]	Week 9[g]	P[e]	Week 11[g]
Nil (n = 19)	434 ± 30	<0.01	311 ± 20	NS	272 ± 19	NS	244 ± 15*	<0.005	271 ± 20
Orange juice (n = 21)	454 ± 29	<0.001	302 ± 18	NS	316 ± 32	NS	324 ± 24	NS	269 ± 23
Folic acid (n = 20)	465 ± 25	<0.001	285 ± 23	NS	292 ± 23	NS	300 ± 22	NS	346 ± 32*
P[f]	NS		NS		NS		< 0.05		< 0.05

[a]Values represent Mean ± SEM

[b]Paired Student's t test, week 0 to 2

[c]Paired Student's t test, week 2 to 5

[d]Unpaired Student's t test, week 5 to 9

[e]Unpaired Student's t test, week 2 to 9

[f]Analysis of variance, Duncan's multiple range test

[g]For each treatment group at week 9 and 11, n = 19

*Significantly different from values in treatment groups at $P < 0.05$

folate in the folic acid group were significantly higher ($P<0.05$) at week 11 than in the other two treatments. A decrease of erythrocyte folate in the orange juice group and increase in both the control and folic acid groups were observed at week 11.

Comparison of Rserum and RRBC values showed identical results to those described here. Serum folates at week 9 were 115, 166 and 215% of week 2 values for the control, orange juice and folic acid groups, respectively. Erythrocyte folates were 84, 112 and 108% of week 2 values for the control, orange juice and folic acid groups, respectively.

4. Effect of oral contraceptives on utilization of folate supplements

Mean serum folates were significantly different between OCA users and nonusers in the nonfolate supplemented controls at week 0 ($P<0.01$), week 2 ($P<0.05$), and week 11 ($P<0.02$) (Table 20). Differences ($P<0.02$) were also observed at week 5 in the folic acid group. Serum folates in OCA users and nonusers were not different from each other at week 9 in any of the three treatment groups. No difference between erythrocyte folates of OCA users and nonusers was detected at any time during the study (Table 21).

Table 20 Effect of oral contraceptives on serum folate of subjects receiving folate supplements

		Serum folate, ng/ml[a]		
		Folate supplement		
Week	Group	Nil (n = 19)	Orange juice (n = 21)	Folic acid (n = 20)
0	OCA users (n = 21)	7.6 ± 0.8	10.5 ± 2.1	7.9 ± 0.7
	Nonusers (n = 39)	11.6 ± 0.9	16.1 ± 3.2	21.5 ± 7.3
	P[b]	<0.01	NS	NS
2	OCA users (n = 21)	6.3 ± 0.6	8.2 ± 1.7	7.2 ± 0.8
	Nonusers (n = 39)	8.4 ± 0.6	10.0 ± 1.2	9.1 ± 1.0
	P[b]	<0.05	NS	NS
5	OCA users (n = 21)	9.1 ± 0.9	15.5 ± 3.7	11.5 ± 1.5
	Nonusers (n = 39)	17.6 ± 6.9	17.2 ± 4.2	32.4 ± 7.5
	P[b]	NS	NS	<0.02
9	OCA users (n = 19)	7.5 ± 0.6	17.6 ± 4.0	10.6 ± 1.3
	Nonusers (n = 38)	8.8 ± 6.9	13.0 ± 0.8	25.1 ± 8.2
	P[b]	NS (n = 19)	NS (n = 19)	NS (n = 19)
11	OCA users (n = 19)	8.1 ± 0.9	27.4 ± 14.1	12.1 ± 0.9
	Nonusers (n = 38)	10.4 ± 0.4	12.3 ± 0.6	28.8 ± 8.3
	P[b]	<0.02 (n = 19)	NS (n = 19)	NS (n = 19)

[a]Values represent Mean ± SEM

[b]Unpaired Student's t test

Table 21 Effect of oral contraceptives on erythrocyte folate of subjects receiving folate supplements

		Erythrocyte folate, ng/ml[a]		
		Folate supplement		
Week	Group	Nil (n = 19)	Orange juice (n = 21)	Folic acid (n = 20)
0	OCA users (n = 21)	478 ± 39	496 ± 24	470 ± 57
	Nonusers (n = 39)	408 ± 42	433 ± 41	462 ± 24
	P[b]	NS	NS	NS
2	OCA users (n = 21)	307 ± 14	290 ± 38	247 ± 13
	Nonusers (n = 39)	314 ± 31	307 ± 21	305 ± 34
	P[b]	NS	NS	NS
5	OCA users (n = 21)	264 ± 22	289 ± 56	273 ± 30
	Nonusers (n = 39)	276 ± 28	329 ± 39	303 ± 32
	P[b]	NS	NS	NS
9	OCA users (n = 19)	230 ± 22	323 ± 50	272 ± 24
	Nonusers (n = 38)	253 ± 21	324 ± 28	313 ± 30
	P[b]	NS (n = 19)	NS (n = 19)	NS (n = 19)
11	OCA users (n = 19)	260 ± 26	266 ± 38	311 ± 49
	Nonusers (n = 38)	277 ± 29	271 ± 29	364 ± 42
	P[b]	NS (n = 19)	NS (n = 19)	NS (n = 19)

[a] Values represent Mean ± SEM

[b] Unpaired Student's t test

V. DISCUSSION

These data support the ready availability of folate from orange juice (Hurdle et al., 1968; Streiff, 1971; Hill et al., 1972; Hoppner et al., 1972; Dong and Oace, 1973), and indicate that this form of biological folate is as available to normal women as is synthetic folic acid (Nelson et al., 1975). These results also confirm that orange juice folate is readily available and absorbed, irrespective of oral contraceptive use.

Serum folate in this study probably reflected folate status more precisely than erythrocyte folate, because of the short period of observation. Erythrocyte folate decreased in the control group over time, reflecting decrease of serum folate during the initial two weeks on the restricted diet. This decrease was prevented by maintaining serum folate with folate supplements, suggesting that the supplemented women were in folate balance. The nonsupplemented women probably were approaching a new lower equilibrium within the normal range, and new, but lower normal levels of erythrocyte folate (Cooper and Lowenstein, 1964).

It is unclear why values at week 11 were not comparable to those of the rest of the study. This was a period immediately prior to Christmas festivities, and alcohol and dietary intake of folate-rich foods may not have been sufficiently restricted. Some subjects reported eating liver pate. Quantitated ethanol intakes by a few subjects showed no significant difference over the study period, but these were very crude measurements and were not representative of true intakes. These data were therefore not considered in the overall interpretation.

In experimental nutritional folate deficiency in man, the serum folate level decreases to below normal within a week of starting a folate-deficient diet and reaches very low levels in three weeks (Herbert, 1962a). The liver of a normal adult contains approximately 7.4 ug/g of total folate (Hoppner and Lampi, 1980), and as the daily requirement for folate is in the region of 50 ug (Herbert, 1962b), the serum folate concentration decreases before there can be significant depletion of body folate stores. The erythrocyte folates become abnormal many weeks after the serum folate level falls below normal (Herbert, 1962a).

Serum and erythrocyte folates in this study behaved in a manner similar to that reported by Herbert (1962a) during the initial two weeks of the folate-restricted diet. Similar decreases were seen in both OCA users and nonusers. Mean serum and erythrocyte folates decreased by 38 and 34%, respectively. The author has no explanation for the rather large drop in erythrocyte folate within two weeks. Generally erythrocyte folate has been more resistant to fluctuations in intake than serum folate. These results suggest an exodus of folate from erythrocytes as no more than 10% of the cells could have been turned over in that short a time.

No effect of oral contraceptives on the utilization of food folate from the mixed diet or the folate supplements was detected, despite striking differences in serum folate levels of OCA users and nonusers at the beginning of the study. The similarity of serum and erythrocyte folates in these women during supervision of their diet, and the similar effect of supplementation with orange juice or folic acid, suggest that lower serum folate levels reported in OCA users (Shojania et al., 1971; Smith et al., 1975) might

reflect different dietary habits rather than interference with folate utilization. However, these OCA users denied dietary intakes different from those of nonusers, so the disparity between folate status in the general unmanipulated population and in those under constant supervision remains to be explained.

The results obtained in this investigation clearly indicate that the folates of orange juice and synthetic folic acid are readily absorbed and utilized. While erythrocyte folate levels decreased significantly ($P<0.005$) in the control group, orange juice and folic acid supplements maintained normal erythrocyte folate and significantly increased serum folate concentrations ($P<0.05$). Full effect was seen at week 9. Erythrocyte folate remained under the levels seen at the inception of the study. Furthermore, serum and erythrocyte folates were significantly lower in the nonfolate supplemented subjects after seven weeks of restricted diet ($P<0.05$). The author calculated that at the end of 120 days, if the fall in erythrocyte folate continued at the same rate, the nonusers not consuming supplements would have a mean erythrocyte folate value of 202 ng/ml. In comparison after the same period, the OCA users with no supplementation would have a mean value of 172 ng/ml. This would indicate a possible folic acid deficiency.

The sudden rise in serum folate during weeks 2 to 5 in both nonfolate supplemented and supplemented subjects is puzzling. Since serum folate is an index of the dietary folate status, these values could represent an intake of folate over and above the restricted diet. However, it is unlikely that all the subjects would falsely report dietary folate consumption at one specific period.

A. The Folate-restricted Diet

The food folate values reported herein are considerably higher than data published for similar foods by Toepfer et al. (1951). This is due to improvements in methodology, primarily with the inclusion of ascorbate in the assay. Direct comparisons with literature are not possible since sampling in this study was done at only one period during the year. This is not expected to cause discrepancies in the evaluation of processed goods, however. The results obtained for drained canned green beans and peas are lower than those of Leichter (1980). The discarding of the liquid portion of canned vegetables entails a significant loss of the vitamin. Wide variation in reported folate content is not unusual since variety and maturity of fruits and vegetables, as well as processing and cooking treatments, differ. Additional losses of folate would be expected during meal preparation and cooking (Cheldelin et al., 1943; Huskisson and Retief, 1970; Taguchi et al., 1973).

Differences in reported results are also due to the methodology of the microbiological analysis. Numerous factors may affect quantification of food folate content: the microorganism used, source and purity of conjugase, extraction methods, pH and incubation time. As well, the variability of values can be attributed to sampling differences in the same product (i.e., leaves, stem, flowers of vegetables).

Food folate values were determined after treatment with conjugase only, as total folate appears to be a more accurate approximation of food folate available to man (Butterworth et al., 1969; Halsted, 1979). Data used in the

preparation of the data tape contained only those literature values determined with Lactobacillus casei and chicken pancreas conjugase. This microorganism responds to a larger spectrum of folate forms than others. Additional foods from those already documented were assayed for folate content because of the limited amount of data in the literature. Also, assay of composites would have been difficult in this study. Rather computer surveys of seven-day dietary intakes were used. Folate values were expressed on the fresh weight of foods as described, because of the large variation reported in cooking effects. The loss of folate activity in cooking was calculated for home-prepared foods, based on change in weight only. Factors permitted adjustment of weights (Merrill et al., 1966).

The folate-restricted diet was nutritionally adequate, providing more than two-thirds of the RDNI for energy and all nutrients tested. Mean intakes did not, however, exceed the RDNI for energy, iron, thiamine and total folate. The lower iron intakes may be due to the elimination of foods which are rich in both folate and iron. Subjects were adequately nourished with respect to protein, vitamin A and vitamin C. The intakes of the latter two nutrients were higher in the folic acid group than in the others, probably because of their larger consumption of both fruit and vegetables. Nutrient intakes were also expressed using the median since it is less influenced by extreme values which occur in distributions of nutrients. Medians cannot be added, and therefore means have been used in estimating intakes.

Calculations of total folate intake excluded folate content of the orange juice and folic acid supplements. The folate-restricted diet

provided much less folate than recommended in the 1980 edition of the Recommended Dietary Allowances of the United States (Food and Nutrition Board, National Research Council, 1980). Only 39% of women were taking diets with 50% of the folate recommended. Folate intakes were also considerably below those recommended in the Canadian standards (Health and Welfare Canada, 1975). No difference was found in total folate intake among the three treatments, however. The subjects in the orange juice group consumed the least folate from the diet, with four women taking less than 100 ug daily. Despite restriction of dietary folate intake, these women consumed on average 159 ± 5 ug/day.

Most studies have not reported the folate content of the diet, because it was either not determined or was ill defined. Food intake and nutritional surveys are seldom used since accurate food tables and adequate microbiological techniques are lacking. A few investigators have assessed the folate contained in the diet of healthy individuals (Butterworth et al., 1963; Chanarin et al., 1968; Santini and Corcino, 1974). Hoppner et al. (1977) calculated that the approximate folate intake from an average daily diet, based on food disappearance data, was 117 ug free folate and 197 ug total folate per person per day. These figures compared favourably with values reported by others (Van de Mark and Wright, 1972; Moscovitch and Cooper, 1973).

The contributions of food groups to folate intake from a normal diet consumed by eight young subjects were documented by Chanarin (1969). In a daily diet consisting of 223 ug free folate, vegetables provided 29% of

the total intake. Cereals supplied 19% and fruit 10% of the daily folate intake. The percent contributions by food groups presented herein are comparable to those mentioned above for fruits and vegetables. Hoppner et al. (1977) estimated a slightly higher (32%) consumption coming from vegetables than was indicated here. This is due to the restriction of some vegetables in this diet. The data are similar to those reported for the Quebec female population of the same age group (Health and Welfare Canada, 1977). Folate intake contributed by the fruit group was lower, however, probably because of the restricted orange juice consumption.

Serum and erythrocyte folates of the 19 nonfolate supplemented women in this study decreased rapidly when foods high in folate were omitted. Although serum and erythrocyte folate values of women taking oral contraceptives were considerably lower than were those of nonusers, levels in both OCA users and nonusers decreased and converged. While on diets which were similar, serum and erythrocyte folates of both OCA users and nonusers remained similar, suggesting that dietary selection rather than a direct effect of the contraceptive medication might be the cause of different levels of folate sufficiency in these women. Despite the low folate intake, serum and erythrocyte folates of almost all of the women not taking folate supplementation were in the normal range. The persistence of normal levels of serum folate in all but one woman, indicates that erythrocyte folate levels would almost certainly equilibrate within the normal range (Cooper and Lowenstein, 1964). This could conceivably indicate that normal folate status could be maintained by the 163 ug/day folate intake. The nine-week period is probably too short to be conclusive, as it took Herbert's subject (Herbert, 1962a) seventeen weeks on a severely restricted diet to achieve

subnormal erythrocyte folate levels. With the mildly restricted intake in the present study, subnormal levels might have appeared in a few months. Few individuals in the general population consume more folate than would be provided by a weekly serving of liver and spinach, and as a limited number of folate-deficiency cases are reported, 200 ug total folate per day would be suggested as the recommended intake. American allowances of 400 ug/day are perhaps set too high.

The mean daily folate intake in this study was almost identical with that reported in a group of pregnant volunteers in whom clinically significant folate deficiency was not observed during pregnancy (Moscovitch and Cooper, 1973). It was similar to the mean folate intake of Canadian nonpregnant women (age 20-39 years; 145 ug/day), calculated by Nutrition Canada based on 24-hour dietary recall data (Health and Welfare Canada, 1977). Based on disappearance data of foods distributed in Canada, Hoppner et al. (1977) have calculated that the typical Canadian diet contains approximately 200 ug of total folate.

B. Hematologic Folate Status of OCA Users

Folate metabolism is altered by orally administered estrogen, but the nature and significance of this change is not clear. Several isolated cases of OCA users with folate deficiency and severe megaloblastic anemia have been described (Paton, 1969; Streiff, 1970; Ryser et al., 1971). Certain underlying disorders in absorption and metabolism may be the principal factors involved in these complications (Toghill and Smith, 1971; Wood et al., 1972).

Conflicting data exist about serum folate concentrations in OCA users. This study confirms the work of others (Shojania et al., 1971; Wertalik et al., 1972; Alperin, 1973; Smith et al., 1975) in demonstrating a significant reduction ($P<0.01$) in the serum folate of women using contraceptives. Shojania et al. (1971) demonstrated a higher urinary FIGLU excretion after histidine loading in OCA users. These abnormalities were time related, and were corrected within three months after discontinuation of contraceptive therapy. Large populations of OCA users with subnormal folate values have also been documented (Luhby et al., 1971; Wertalik et al., 1972; Whitehead et al., 1973; Smith et al., 1975).

None of the subjects in this study had folate values which were subnormal. In fact, values were considerably higher in nonusers than was found by other investigators (Spray, 1968; McLean et al., 1969; Shojania et al., 1971; Stephens et al., 1972; Paine et al., 1975; Pietarinen et al., 1977). Serum folates of OCA users were more consistent with these reports. Mean erythrocyte folates in two Canadian studies (Shojania et al., 1971;

Pietarinen et al., 1977) were 160 ng/ml for OCA users, and 190 ng/ml for the control group. The values reported herein were again considerably higher than those mentioned above.

Serum folate has been known to progressively fall with continued contraceptive ingestion. The percentage of subjects with low serum folates increases with the duration of oral contraceptive therapy (Shojania et al., 1971). It may be that the subjects in this study were not consuming contraceptives for a sufficient period of time to demonstrate a substantial effect. Only three had been taking oral contraceptives for more than three years.

The higher serum and erythrocyte folates are probably due to dietary intake. Most subjects enjoyed fresh vegetables and salads, and especially at the time when this study was initiated, this particular produce was abundant. Most reports have not given extensive description of the food folate patterns of their subjects. Shojania et al. (1971) performed their investigation in Winnipeg, Manitoba, an area characterized by long, cold winters and very short summers, with the consequence that consumption of vegetables and fruit is small. It is assumed that many had a low dietary intake, and consequently lower serum and erythrocyte folate concentrations. Pietarinen et al. (1977) stated that although the dietary folate intakes were not significantly different between their OCA users and nonusers, the serum folate levels were significantly lower in women taking oral contraceptives.

The mechanism responsible for this decrease in serum folate has not been delineated. It has been suggested (Necheles and Snyder, 1970; Streiff,

1970) that the sex steroid hormones may affect intestinal absorption of food folates by inhibiting conjugase activity. Since monoglutamate and polyglutamate absorption is unaltered in OCA users presaturated with folic acid, it is unlikely that this is responsible for decreased serum folates (Stephens et al., 1972; Shojania and Hornady, 1973). Therefore oral contraceptives may increase plasma folate clearance.

A number of groups have not confirmed the findings of lower serum folate concentrations in women taking oral contraceptives (Spray, 1968; McLean et al., 1969; Maniego-Bautista and Bazzano, 1969; Kahn et al., 1970; Pritchard et al., 1971; Stephens et al., 1972; Paine et al., 1975). It is possible that the variation in results between this study and others can be explained by differences in populations studied, in nutritional status of the subjects, in hormonal content of various contraceptives, or in duration of contraceptive therapy. These discrepancies are also probably attributable to differences in assay procedures, fasting or nonfasting state of the subjects, day of menstrual cycle at which blood samples are drawn, and individual variations in metabolic handling of folate. Shojania et al. (1971), in their criticism of these reports, stated that the sample number was too small, control subjects were not all from the same source, and the duration of oral contraceptive therapy was too short. They tried to exclude from their study many of the factors which could have been responsible for the failure of others to recognize impaired folate metabolism in OCA users. Their subjects were from the same socioeconomic group and age bracket, and were interviewed regarding their diets and medications.

The sample size in this study was not as large as some reported, but was large enough to make a meaningful statistical analysis. However, too few subjects were receiving long term oral contraceptive therapy to see the accumulated effect on folate metabolism.

On the other hand erythrocyte folate concentrations, which are more indicative than serum folate of body folate stores, were not significantly different between OCA users and nonusers. Levels were initially higher in OCA users, however. Five percent of these women routinely used vitamin-mineral supplements before entering the study. These results are in contrast to the work of others (Shojania et al., 1971; Alperin, 1973; Prasad et al., 1975; Smith et al., 1975) who found a decrease in erythrocyte folate levels of OCA users. Prasad et al. (1975) showed a definite lowering effect of contraceptives in serum and erythrocyte folates in subjects of the upper socioeconomic group. In the lower strata, both serum and erythrocyte folates were lower than in the upper socioeconomic group of subjects, indicating that this population may be marginally folate deficient. Although this hypothesis could apply to the serum parameter in these subjects, it does not explain the effect of contraceptives in this socioeconomic group. Spray (1968) and Pietarinen et al. (1977) found that erythrocyte folates of OCA users and nonusers were similar. It may be that the duration of contraceptive use is also a factor in determining erythrocyte folate levels.

Variations in reported folate levels may also be due to the time of sampling during the menstrual cycle. Stephens et al. (1972) demonstrated that fasting serum folate did not significantly alter with the different phases of the normal menstrual cycle, either in OCA users or nonusers.

Pietarinen et al. (1977) showed that significant differences existed only at day 5 and not day 20. Although the author was aware of the possible effect of endogenous sex hormones on folate status, difficulty existed in scheduling subjects for specific days. Blood was taken during periods when subjects were taking oral contraceptives.

C. Availability of Orange Juice Folate

Differences in reported folate content of orange juice are due to the variety and maturity of the fruit, growth season, source, processing technique, form and brand name, presence of pulp, dilution, and assay procedures. There is also some variation in folate levels between the individual fruit. The total folate concentration in this reconstituted frozen orange juice agrees with four other reports in which a similar assay technique was employed (Streiff, 1971; Hill et al., 1972; Hoppner et al., 1972; Dong and Oace, 1973), but is considerably higher than results of earlier studies (Krehl and Cowgill, 1950; Toepfer et al., 1951; Teply et al., 1953; Burger et al., 1956; Herbert, 1963). Malin (1974) found that the pH specificity of the chicken pancreas conjugase may affect total folate content dramatically. No polyglutamate was detected at natural pH 3.6 in frozen reconstituted orange juice concentrate. Adjustment of the juice to 7.6 before conjugase hydrolysis gave a total folate content of 47.7 ug/100 ml. The free folate level was 22.4 ug/100 ml of juice. Since orange juice contains an appreciable quantity of folate, it has been thought to be a good source of this folate.

Streiff (1971) and Tamura and Stokstad (1973) showed that the majority of orange juice folates were of the free form. Butterfield and Calloway (1972) and Dong and Oace (1973) found that, without conjugase treatment, not all folate activity was being tested. When Tamura et al. (1976) separated the folates of orange juice by column chromatography, predominantly reduced methylated polyglutamates were detected. They suggested that the variation in results between the microbiological and chromatographic

assays was due to the large spectrum of folate forms utilized by Lactobacillus casei. Since monoglutamates and polyglutamates are present in orange juice, it would be expected that these folates are available for absorption and utilization (Cooper, 1977; Bertino et al., 1977).

Previous reports (Tamura and Stokstad, 1973; Tamura et al., 1976) that the folate in orange juice concentrates was not readily available and that these concentrates impeded utilization of other folate in the diet, suggested that this was probably affected by the use of undiluted concentrate, which may affect folate utilization by pH or another effect. Those studies also required much larger folate concentration presented to the intestinal lumen than probably is presented by the normal diet, and might have tested a transport system with different affinity for folate.

This author chose to study the bioavailability of orange juice folate by monitoring serum and erythrocyte folate concentrations. Even though this technique has been questioned because of the difficulty in quantifying change, a marked rise was seen in the serum folate in this study. Tamura et al. (1976) measured urinary folate excretion in presaturated subjects, and compared it to other folates. They found that this technique permitted estimation of the folate absorbed, even though low levels of folate may be present in some foods. Nelson et al. (1975) used the triple lumen perfusion system to demonstrate availability of orange juice folate with respect to synthetic folic acid. Colman and Herbert (1976) criticized their report on the basis of unlabeled bile folate present in the segment of the bowel under study.

As man cannot synthesize the pteridine structure, he must depend on dietary intake for his natural source of folates. Although many folate-rich foods exist, their folates are often depleted in cooking or processing. A stable, easy-to-prepare, and inexpensive source of folate is needed, which does not require cooking prior to ingestion. Orange juice is one of the most popular foods, with a generally appealing aroma and a bonus of significant nutrition. It is a good, readily available source of folate, providing about 20% of the RDNI in a 100 to 250 ml serving. Orange juice, either in the fresh or reconstituted frozen concentrate form, appears to be an excellent source of dietary folate. For people with increased folic acid requirements, such as women with low serum folate taking oral contraceptives, orange juice could be a source of supplementation. Those who depend on frozen orange juice for their daily vitamin C would also be satisfying their folic acid needs.

Many areas still need to be explored. A follow-up study of longer duration, larger sample size and more complete food folate values could decisively test the availability of folate from orange juice. Further research could evaluate the folic acid nutritional status in population groups or in clinical patients suspected of increased folic acid requirements. The benefit of folate fortification with orange juice could be discerned. Well-controlled studies of the effects of oral contraceptive treatment on the folic acid status of populations with inadequate nutritional standards would be of great interest. Lastly, tables of folic acid content of foods and dietary information on folate intakes should be compiled to alleviate the inadequacy of available information.

VI. CONCLUSION

These data confirm that folate in reconstituted orange juice is equal in bioavailability to synthetic folic acid in this population of normal women. Utilization of both folate forms and folate in the mixed diet was unaffected by oral contraceptive medication. The results also indicate that a much lower daily folate intake than currently recommended should be adequate for healthy women.

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APPENDIX A

SUPPLEMENTARY INFORMATION FOR

III. MATERIALS AND METHODS

Appendix Table Ai. Dietary questionnaire

NAME _____
Family First Initials

ADDRESS _____
Home

_____
Business

TELEPHONE Home _____ BUSINESS _____
HEIGHT _____ WEIGHT _____ YEAR OF BIRTH _____
MARITAL STATUS _____ NATIONALITY _____

Are you living at home? _____ Institution (describe) _____
Do you own a freezer or have
one at your disposal? _____ Is it self-defrosting? _____

Name the stores which are most frequented by you:

1. Name _____
   Location _____
2. Name _____
   Location _____
3. Name _____
   Location _____

Have you ever suffered from anemia? __ What kind? ___ Other blood disorders?
If so, when? _____ _____

Do you use oral contraceptives? _____ Since when? _____

What kind of oral contraceptive do you use?
Brand name _____

Dosage of hormones Progesterone _____

Estrogen _____

Do you regularly consume alcohol? _____ How much per week? _____
What kind of alcoholic beverages do you drink? _____
Do you take vitamins? _____ What kind? _____
Would you be willing to discontinue taking vitamins for the test? _____
Would you be willing to drink orange juice (frozen concentrate) daily? _____
Would you be willing to consume a folic acid supplement daily? _____
Are you willing to give up high folic acid foods for the duration of the study? _____
Are you willing to do without alcoholic beverages? _____
If not, are you willing to record the amount consumed? _____
Would you be willing to serve as a volunteer? _____

RAILWAY STATIONS AND EXPRESS
(C.N.R. & C.P.R.) AND TELEGRAPHIC
ADDRESS. STE ANNE DE BELLEVUE. QUE.

POST OFFICE ADDRESS:
MACDONALD COLLEGE, QUE., CANADA

MACDONALD CAMPUS
OF
McGILL UNIVERSITY

September 16, 1977

From: Barbara M. Rhode, Graduate student
Macdonald College, School of Food Science
Ste. Anne de Bellevue, Quebec H0A 1C0
Telephone number School 457-3123 or 457-6580 local 377
Home 482-4891

Dear

Thank you very much for having decided to be a participant in this research. If at any time during the 3½-month period you have questions or wish to discuss any aspect of the study, please feel free to contact me at any one of the above telephone numbers. Although I shall only be seeing you on blood sampling days and at those times when supplements will be given, I still in fact would like to communicate with you and therefore encourage you to telephone me if there is the slightest difficulty.

To recap again and highlight the points which should be kept in mind during the course of the study, they are as follows:
(1) All subjects will remain on the moderately-low folate diet, irrespective of whether or not folate supplementation is involved. All foods are permitted on this diet, with the exception of LIVER, KIDNEY, SPINACH, ORANGES, TANGERINES and ORANGE JUICE. Eggs have been restricted to 3 per week. The consumption of vitamins is not permitted, and alcohol intake, in terms of kind and quantity, is left up to the discretion of the participant. I ask, however, that the amount consumed be recorded during the week of the diet survey. The subjects start following this diet the day of their first blood sample.
(2) There will be 2 7-day weighed/measured diet surveys done. This "exercise" is necessary so that a consumption pattern can be established and folic acid values for various Canadian foods can be obtained. For this reason, it is NECESSARY to keep the diet during the surveys as similar as possible to the one consumed during the other 3 months. As well, I ask that EVERYONE try to be ACCURATE in their recording; in terms of the description of food, the form it was served in (boiled, baked, raw) and the weighed or measured amount eaten.
(3) The supplements should be prepared and taken as instructed. They, as well as the scales, should be picked up from a prearranged area.
(4) All should be punctual for the blood sampling and come there in a fasted state.
(5) The days of menstruation should be recorded for the months of September, October, November and December.

Thanks very much once again.

Yours sincerely,

Barbara M. Rhode

Appendix Table Aiii. Instructions for administration of supplements

ORANGE JUICE SUPPLEMENTATION

Place the frozen concentrate in the freezer immediately and keep frozen at 0^{o}F until ready to use. If freezing unit fails and juice thaws, use immediately.

To reconstitute juice, place can under water tap to speed thawing or leave unopened at room temperature for a short time. Follow instructions on can (1 6 oz. can frozen concentrate + 3 full 6 oz. cans cold tap water). Prepare one can at a time.

Always store the juice in a covered container (amber jar) in the refrigerator.

Shake or stir before drinking to aerate the juice and bring out peak flavour.

Drink 5 ounces of the reconstituted orange juice in the morning and 5 ounces in the evening. At all times, drink all the juice down to the last drop.

Under no circumstances is the use of orange juice permitted by individuals NOT involved in the study. This is a special orange juice (it is all from the same batch) and should therefore not be substituted by any other brand, can or package of orange juice or drink.

You will get 4 6 oz. cans of frozen concentrate per week. Please pick up a fresh supply every Wednesday from the Vitamin Lab, MDD, School of Nursing or MAC.

FOLIC ACID SUPPLEMENTATION

The folic acid solution will be given to you in amber jars. Keep it covered and stored under refrigeration at all times. Do not expose to light.

Syringes will be provided. Use a NEW one each time the supplement is taken.

Measure out 0.5 ml in the morning and 0.5 ml in the evening, to make a total of 1.0 ml consumed during the span of one day.

Deposit the 0.5 ml of folic acid solution into 4 ounces of tap water and release the plunger several times, drawing water up into the barrel and pushing it out again. This ensures that the folic acid is completely washed into the water. THROW OUT THE SYRINGE. Drink the liquid and fill up the glass again with an equal volume of water. Drink this and swish some of the liquid in your mouth to wash any residual folic acid down.

Please pick up a fresh supplement every Wednesday and RETURN THE AMBER JAR AT THE SAME TIME. There should still be some of the solution left. YOU WILL BE HELD RESPONSIBLE FOR THE CONDITION OF THE JARS.

Under no circumstances is the use of the folic acid supplement or syringes permitted by individuals NOT involved in the study.

Appendix Table Aiv. Informed consent statement

I hereby agree to be a participant in the folic acid study for a period of approximately 3 months, from the end of September to the middle of December in the year 1977 and agree to comply with the following conditions, those being

(1) to remain on the moderately-low folate diet (no liver, kidney, spinach, oranges, tangerines, orange juice, vitamins, restricted quantity of eggs) for the duration of the study

(2) to remain in the preassigned treatment group for the duration of the study

(3) to prepare, store and take the supplements as instructed

(4) to avoid permitting non-participants the consumption of the "supplied" orange juice or folic acid supplement

(5) to return all scales, computer booklets and amber jars in the same condition as they were on receipt and to pay for any damage to the stated equipment

(6) to be punctual when coming for blood samples and be there in a fasted state

(7) to give 5 14.5 ml samples of blood over the 3 months

(8) to pick up the supplements, scales, booklets or notices from a prearranged area

(9) to complete the 2 7-day diet surveys truthfully and to the best of my ability and try and make it as typical of my diet as possible (alcohol included)

(10) to record the days of menstruation from September to December

In return I am to receive $25.00 ON COMPLETION OF THE STUDY.

Date _____ Signature _____

Name Print _____

Appendix Table Av. Report of individual serum and erythrocyte folate status at blood sampling weeks

027 TEST GROUP ----------

HEMATOLOGIC STATUS OVER A 3-MONTH PERIOD--STUDY
ON THE BIOAVAILABILITY OF FOLIC ACID (FA)
FROM FROZEN ORANGE JUICE CONCENTRATE.
(SEPTEMBER-DECEMBER 1977)

I.D. NO.	WEEK OF BLOOD SAMPLING	SERUM FA NG/ML	RBC FA NG/ML	WHOLE BLOOD FA NG/ML	HCT %
027	00	011.8	259	192	39
027	02	009.4	316	216	38
027	05	005.9	302	208	39
027	09	006.8	230	176	38
027	11	010.4	243	184	36

VALUES FOR SERUM AND RED BLOOD CELL FOLATE ARE GIVEN FOR THE DIFFERENT BLOOD SAMPLINGS WHICH WERE TAKEN DURING THE WEEKS INDICATED.

SERUM FOLATE IS A MEASUREMENT OF THE FOLIC ACID WHICH HAS BEEN ABSORBED OVER A 3-DAY PERIOD. IT IS AN INDICATION OF YOUR DIETARY FOLATE STATUS. LEVELS OF LESS THAN 4 NG/ML CONSTITUTE A DEFICIENCY STATE.

RED BLOOD CELL (RBC) FOLATE IS A MEASUREMENT OF THE FOLATE PRESENT IN THE BODY OVER THE LAST 120 DAYS (THE TIME IT TAKES TO MANUFACTURE A MATURE RBC). IT IS AN INDICATION OF YOUR BODILY FOLATE STATUS AND IS USED AS AN INDEX IN TESTING FOR DEFICIENCIES. LEVELS OF LESS THAN 175 NG/ML CONSTITUTE A DEFICIENCY STATE.

THE HEMATOCRIT (HCT) IS THE PERCENTAGE OF THE VOLUME OF BLOOD OCCUPIED BY RED BLOOD CELLS. THE AVERAGE HCT FOR WOMEN IS 42%.

Appendix Table Avi. Sample page illustrating nutrient composition of USDA foods from manual

FOOD	NAME PER 100G. E.P.	WATER %	KCAL	PROT G	FAT G	CA MG	IRON MG	VITA I.U.	THIAM MG	RIBO MG	VITC MG	FIBRE G
1323	MILK 2%FAT	87.0	59	4.2	2.0	143	0.1	80	0.04	0.21	1	0.0
1324	MILK CN EVAP UNSW	73.6	137	7.0	7.9	252	0.1	320	0.04	0.34	1	0.0
1325	MILK CN COND SW	27.1	321	8.1	8.7	262	0.1	360	0.08	0.38	1	0.0
1327	MILK DRY SKIM REG	3.0	363	35.9	0.8	1308	0.6	30	0.35	1.80	7	0.0
1328	MILK DRY SKIM INS	4.0	359	35.8	0.7	1293	0.6	30	0.35	1.78	7	0.0
1329	MILK MALTED DRY	2.6	410	14.7	8.3	288	2.1	1020	0.33	0.54	0	0.3
1330	MILK MALTED BEV	78.2	104	4.7	4.4	135	0.3	250	0.06	0.21	1	0.0
1331	MILK CHOC BEV COM SM	82.8	76	3.3	2.3	108	0.2	80	0.04	0.16	1	0.0
1332	CHOC DRINK	81.5	85	3.4	3.4	111	0.2	130	0.03	0.16	1	11.0
1333	MILK CHOC BEV HOT HR	80.5	95	3.3	5.0	104	0.2	140	0.03	0.16	1	0.1
1334	MILKCOCOA BEV HOT HR	79.0	97	3.8	4.6	116	0.4	160	0.04	0.18	1	0.1
1338	MILLET HG	11.8	327	9.9	2.9	20	6.8	0	0.73	0.38	0	3.2
1339	MOLASSES LIGHT	24.0	252	0.0	0.0	165	4.3	0	0.07	0.06	0	0.0
1340	MOLASSES MEDIUM	24.0	232	0.0	0.0	290	6.0	0	0.00	0.12	0	0.0
1341	MOLASSES BLACKSTRAP	24.0	213	0.0	0.0	684	16.1	0	0.11	0.19	0	0.0
1342	MOLASSES BARBADOS	24.0	271	0.0	0.0	245	0.0	0	0.06	0.20	0	0.0
1343	MUFFINS PLAIN ENR HR	38.0	294	7.8	10.1	104	1.6	100	0.17	0.23	0	0.1
1345	MUFFINS HR BLUEBRRY	39.0	281	7.3	9.3	84	1.6	220	0.16	0.20	1	0.5
1346	MUFFINS ENR BRAN HR	35.1	261	7.7	9.8	142	3.7	230	0.14	0.24	0	1.8
1347	MUFFINS CORN ENR HR	32.7	314	7.1	10.1	105	1.7	300	0.20	0.23	0	0.2
1349	MUFFIN MIX DRY ENR	7.6	417	6.2	11.5	300	1.8	150	0.24	0.16	0	0.5
1350	MUFFIN MIX EGG MILK	50.4	324	6.9	10.6	241	1.5	240	0.18	0.19	0	0.2
1354	MUSHROOM COM RAW	90.4	28	2.7	0.3	6	0.8	0	0.10	0.46	3	0.6
1355	MUSHROOM # COM CN	93.1	17	1.9	0.1	6	0.5	0	0.02	0.25	2	0.6
1356	MUSHROOM NONCOM RAW	89.1	35	1.9	0.6	13	1.4	0	0.10	0.33	3	1.1
1357	MUSKELLUNGE RAW	76.3	109	20.2	2.5	0	0.6	0	0.00	0.00	0	0.0
1358	MELON R CANTALOUP	91.2	30	0.7	0.1	14	0.4	3400	0.04	0.03	33	0.3
1360	MELON R HONEYDEW	90.6	33	0.8	0.3	14	0.4	40	0.04	0.03	23	0.3
1361	MELON BALLS FR SRP	83.2	62	0.6	0.1	10	0.3	1540	0.03	0.02	16	0.3
1364	MUSSELS RAW MEAT**	78.6	95	14.4	2.2	88	3.4	0	0.16	0.21	0	0.0
1372	MUSTARD PREPARE BR	78.1	91	5.9	6.3	124	1.8	0	0.00	0.00	0	1.3
1373	MUSTARD PREPARE Y	80.2	75	4.7	4.4	84	2.0	0	0.00	0.00	0	1.0
1374	NECTARINES RAW	81.8	64	0.6	0.0	4	0.5	1650	0.00	0.00	13	0.4
1375	NEWZEALAND SPINACH R	92.6	19	2.2	0.3	58	2.6	4300	0.04	0.17	30	0.7
1376	NEWZEALAND SPINACH C	94.8	13	1.7	0.2	48	1.5	3600	0.03	0.10	14	0.6
1377	NOODLES EGG ENR DRY	9.8	388	12.8	4.6	31	2.9	220	0.88	0.38	0	0.4
1378	NOODLES EGG ENR COOK	70.4	125	4.1	1.5	10	0.9	70	0.14	0.08	0	0.1
1381	NOODLES CHOW MEIN CN	1.1	489	13.2	23.5	0	0.0	0	0.00	0.00	0	0.0
1384	OAT FLAKES INS N DRY	7.4	384	14.6	4.2	50	3.5	0	0.35	0.00	0	0.7
1385	OAT FLAKES INS N C	83.0	69	2.6	0.8	10	0.6	0	0.05	0.00	0	0.1
1386	OAT GRANULE QC N DRY	7.0	383	14.8	4.0	60	3.8	0	0.40	0.00	0	1.1
1387	OAT GRANULE QC C N	85.2	60	2.3	0.6	10	0.6	0	0.06	0.00	0	0.2
1390	ROLLED OATS N DRY	8.3	390	14.2	7.4	53	4.5	0	0.60	0.14	0	1.2
1391	ROLLED OATS C N	86.5	55	2.0	1.0	9	0.6	0	0.08	0.02	0	0.2
1392	OATS PROT+NUTR U	3.9	379	18.8	2.1	265	5.3	0	3.53	4.23	0	1.8
1393	OATS PUFF NUTR U	3.4	397	11.9	5.5	177	4.7	0	0.98	0.18	0	1.1
1394	OATS PUFF NUTR U SG	1.9	396	6.7	3.4	72	4.4	0	1.03	0.12	0	0.7
1395	OAT FLAKE NUTR U SLY	3.5	397	14.9	5.7	150	8.5	0	0.71	0.33	0	0.9
1396	OCEAN PERCH ATLA RAW	79.7	88	18.0	1.2	20	1.0	0	0.10	0.08	0	0.0
1397	OCEAN PERCH ATLA C	59.0	227	19.0	15.3	33	1.3	0	0.10	0.11	0	0.0
1398	OCEANPERCH ATLA FR C	43.2	319	16.9	18.9	0	0.0	0	0.00	0.00	0	0.0
1401	OIL SALAD OR COOKING	0.0	884	0.0	100.0	0	0.0	0	0.00	0.00	0	0.0
1402	OKRA RAW	88.9	36	2.4	0.3	92	0.6	520	0.17	0.21	31	1.0
1403	OKRA C BOILED DRAIN	91.1	29	2.0	0.3	92	0.5	490	0.13	0.16	20	1.3
1404	OKRA FR CUTS & PODS	87.9	39	2.3	0.1	94	0.6	480	0.17	0.21	16	1.0

APPENDIX B

SUPPLEMENTARY STATISTICAL ANALYSES

List of Abbreviations

Abbreviation	Meaning
T	Week of blood sampling
Diet	Treatment groups 1: Nil 2: Orange juice 3: Folic acid
OCA	Oral contraceptive groups 1: Non OCA users 2: OCA users
Group	Experimental group 1: Non OCA users nil group 2: Non OCA users orange juice group 3: Non OCA users folic acid group 4: OCA users nil group 5: OCA users orange juice group 6: OCA users folic acid group
Rserum	Ratio of serum folate at sampling period to that at week 2
RRBC	Ratio of erythrocyte folate at sampling period to that at week 2
n	Number of subjects

a. Nutrient data

Appendix Table Bai. Nutrient intakes of individual subjects during two dietary survey weeks

S T A T I S T I C A L A N A L Y S I S S Y S T E M 1

OBS	SUBJECT	CAL	PROT	FAT	CALCIUM	IRON	VITA	THIAM	RIBO	VITC	TFOLATE	DIET	DCA
1	30	1973.1	74.21	73.80	1231.1	10.54	10476.9	1.11	1.97	145.9	228.0	1	1
2	27	1567.7	54.45	67.59	264.5	11.20	4972.9	0.93	0.86	32.9	132.1	1	1
3	32	2362.3	86.73	122.68	1230.0	13.49	6487.3	1.88	2.28	113.7	186.4	1	1
4	10	1691.4	61.29	63.27	695.1	9.36	6613.7	0.75	1.34	66.4	157.5	1	1
5	23	1799.2	77.73	103.36	522.8	12.60	9417.9	1.50	1.13	93.0	173.7	1	1
6	3	1533.2	63.29	67.10	1023.2	8.85	3197.0	0.95	1.62	28.7	116.8	1	1
7	5	1850.9	72.87	78.25	1744.9	11.23	5491.4	0.83	1.62	66.6	150.8	1	1
8	17	1179.7	52.79	41.23	549.0	7.93	5128.4	0.79	1.19	55.7	110.2	1	1
9	30	1345.2	85.61	56.41	1371.2	14.16	10939.1	1.25	2.11	146.3	257.4	1	1
10	10	1756.9	77.01	74.59	702.0	12.45	5925.4	1.76	1.60	75.7	209.2	1	1
11	5	1534.9	59.10	70.91	878.7	10.31	4265.6	0.66	1.39	77.2	122.4	1	1
12	23	1139.2	50.38	61.19	525.1	8.74	3852.4	0.92	0.77	56.2	122.0	1	1
13	31	1844.7	82.66	67.93	895.1	13.80	6575.4	1.33	2.05	99.4	188.5	1	1
14	27	1523.0	57.03	72.13	527.9	8.52	3163.9	1.05	1.25	71.4	153.6	1	1
15	3	1318.6	61.89	49.82	738.2	9.48	2649.7	0.93	1.37	64.6	138.0	1	1
16	32	2371.1	77.73	121.09	1028.0	14.04	5798.9	1.53	2.15	101.4	219.9	1	1
17	1	2065.3	82.41	82.53	871.1	11.48	2752.4	1.05	1.63	42.4	177.4	1	2
18	14	1537.4	62.86	58.88	725.2	9.81	3547.7	1.11	1.23	55.2	127.7	1	2
19	14	1633.4	68.55	62.92	841.2	10.57	3622.9	0.77	1.39	42.5	154.3	1	2
20	1	1857.4	82.38	79.52	985.5	11.37	2868.8	0.99	1.61	49.3	139.8	1	2
21	29	1676.8	79.01	68.77	930.3	12.08	6051.8	0.92	1.63	86.0	218.0	2	1
22	26	1410.8	56.42	41.69	648.0	8.68	3788.2	0.68	1.19	53.1	137.6	2	1
23	7	1722.0	66.17	62.23	906.5	12.20	4868.1	1.02	1.75	98.0	207.9	2	1
24	54	1627.2	61.91	81.75	595.7	9.31	3023.4	1.01	1.36	38.3	125.3	2	1
25	6	2104.8	105.20	80.91	1210.1	15.34	7927.1	1.66	2.22	87.0	197.4	2	1
26	22	1335.9	45.22	62.76	361.7	7.75	2549.6	0.82	0.83	37.5	71.2	2	1
27	8	1484.5	54.96	63.41	686.3	10.78	4704.3	1.04	1.27	75.2	202.8	2	1
28	21	1310.0	69.35	71.35	611.4	8.52	4678.5	0.83	1.41	32.9	84.0	2	1
29	19	1325.7	59.77	48.40	512.9	9.54	5982.6	0.99	1.15	78.8	194.8	2	1
30	12	1372.0	53.66	85.98	107.5	9.53	2515.5	1.07	0.74	49.8	83.5	2	1
31	53	1493.5	72.76	65.53	889.3	13.81	3847.5	1.46	2.15	72.4	161.7	2	1
32	29	1380.2	75.85	52.13	1362.0	10.34	8700.3	0.79	1.92	56.4	182.5	2	1
33	17	1675.3	83.28	59.36	660.4	13.54	3200.5	1.19	1.62	113.3	200.7	2	1
34	19	2779.2	93.62	97.77	823.1	11.73	4727.2	1.69	1.78	79.2	234.6	2	1
35	22	1242.1	47.36	68.71	493.9	6.73	5088.9	0.51	0.99	24.7	100.5	2	1
36	21	1486.1	67.87	65.12	703.2	10.43	2670.1	1.19	1.55	44.4	119.4	2	1
37	7	1621.2	65.46	69.30	1048.9	10.68	8092.5	0.91	1.44	56.8	200.5	2	1
38	54	1494.0	52.59	72.53	603.2	8.01	2144.7	0.84	1.19	40.3	119.5	2	1
39	53	1457.2	56.78	74.00	722.4	9.58	5804.9	0.90	1.43	27.1	104.6	2	1
40	6	2130.3	76.73	93.07	1091.7	11.69	9969.5	1.06	1.67	40.4	177.4	2	1
41	8	1660.6	61.87	60.97	756.6	11.21	4377.7	1.19	1.34	100.0	166.3	2	1
42	12	981.1	43.21	46.70	223.5	8.57	3197.6	1.00	0.73	46.6	82.0	2	1
43	52	2838.6	98.90	172.86	793.9	15.36	4815.6	1.66	1.89	212.7	203.4	2	2
44	33	1864.3	71.89	77.15	727.1	11.45	2170.4	1.06	1.67	55.5	130.3	2	2
45	16	1603.7	82.07	63.70	1057.5	9.90	4498.0	0.77	1.75	32.2	170.8	2	2
46	24	2013.8	74.31	106.44	727.7	10.79	4245.6	0.69	1.34	30.5	96.8	2	2
47	24	1717.2	70.61	78.71	806.7	11.91	6175.7	0.77	1.29	64.7	126.0	2	2
48	16	1786.7	84.95	79.15	1141.5	10.43	3224.1	1.38	1.58	81.5	137.8	2	2
49	33	1715.8	63.18	60.49	756.3	10.46	3199.9	1.03	1.52	47.3	132.4	2	2
50	52	2158.1	69.25	118.95	879.0	11.17	5419.0	1.30	1.57	103.5	148.6	2	2
51	4	1493.3	62.29	68.05	642.2	10.80	6497.9	0.76	1.06	107.4	160.1	3	1
52	59	2731.7	94.61	142.92	633.5	16.49	6283.8	1.48	1.78	145.7	197.6	3	1
53	20	2013.7	84.82	90.11	1275.5	10.13	6193.8	0.93	2.14	61.3	154.3	3	1

B4 contd.

continued

Appendix Table Bai (continued)

STATISTICAL ANALYSIS SYSTEM 2

OBS	SUBJECT	CAL	PROT	FAT	CALCIUM	IRON	VITA	THIAM	RIBO	VITC	TFOLATE	DIET	OCA
54	28	1494.2	77.75	67.84	819.4	11.11	7034.9	1.33	1.47	63.6	136.4	3	1
55	9	1551.4	66.58	58.92	648.1	12.22	4707.2	0.92	1.39	34.8	200.7	3	1
56	15	1405.1	72.48	54.37	857.2	10.57	11985.7	0.89	1.48	71.7	152.0	3	1
57	13	1781.1	86.80	65.59	540.4	12.36	8675.1	0.96	1.44	123.0	232.4	3	1
58	2	1746.4	85.59	66.89	1088.2	8.44	5492.1	1.08	2.41	95.6	156.3	3	1
59	13	1323.5	63.27	55.44	466.9	10.18	7984.4	1.12	1.19	141.5	178.5	3	1
60	15	1045.0	43.19	43.95	738.4	7.05	8978.6	0.66	1.10	71.7	126.2	3	1
61	20	2102.1	81.21	78.46	1191.8	10.20	3911.6	1.20	2.16	66.4	154.7	3	1
62	2	2149.8	104.43	98.87	1535.0	10.63	5081.9	1.17	2.64	112.6	217.8	3	1
63	4	1368.3	53.66	61.34	691.6	9.84	5551.9	0.74	1.08	95.3	153.3	3	1
64	28	1735.5	93.25	71.95	925.5	13.10	9043.5	1.79	1.76	115.4	195.8	3	1
65	59	2112.4	84.74	100.45	743.3	14.97	5410.4	1.41	1.59	112.6	192.9	3	1
66	9	1374.2	47.61	56.87	446.2	9.34	3793.6	1.24	1.04	84.3	125.8	3	1
67	11	2139.9	85.54	84.59	1046.7	14.19	4464.7	1.79	2.19	133.0	193.7	3	2
68	25	1904.8	65.58	95.81	614.1	10.64	2708.9	0.82	1.06	47.6	134.5	3	2
69	11	1543.3	63.40	57.48	976.9	10.35	5411.1	1.10	1.74	102.7	182.1	3	2
70	25	1849.9	59.17	75.19	743.7	9.54	4617.3	0.94	1.44	40.0	126.0	3	2

Appendix Table Bii. Multivariate analysis of variance of the effects of diet and OCA on energy intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 4

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: CAL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	861425.27321112	287141.75773704	2.37	0.0772	0.097272	20.4831
ERROR	66	7994426.94521810	121127.68098815		STD DEV		CAL MEAN
CORRECTED TOTAL	69	8855852.21842922			348.03402275		1699.12714286

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	91468.41742855	0.38	0.6870	2	135383.03015296	0.56	0.5746
OCA	1	769956.85578257	6.36	0.0141	1	769956.85578257	6.36	0.0141

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE CAL

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=121128

GROUPING	MEAN	N	GROUP
A	1902.275000	8	5
A A			
B A	1859.475000	4	6
B A			
B A	1760.875000	4	4
B A			
B A	1714.231250	16	3
B A			
B A	1703.193750	16	1
B			
B	1549.113636	22	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=121128

GROUPING	MEAN	N	DIET
A	1743.280000	20	3
A A			
A A	1714.730000	20	1
A A			
A	1659.290000	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=121128

GROUPING	MEAN	N	OCA
A	1886.225000	16	2
B	1643.690741	54	1

Appendix Table Baiii. Multivariate analysis of variance of the effects of diet and OCA on protein intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 5

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: PROT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	566.86236598	188.95412199	0.85	0.4732	0.037259	21.1074
ERROR	66	14647.12695402	221.92616597		STD DEV.		PROT MEAN
CORRECTED TOTAL	69	15213.98932000			14.89718651		70.57800000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	239.47710333	0.54	0.5856	2	263.19416010	0.59	0.5556
OCA	1	327.38526264	1.48	0.2289	1	327.38526264	1.48	0.2289

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE PROT

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=221.926

GROUPING	MEAN	N	GROUP
A	77.570000	8	5
A A	74.767500	16	3
A A	74.055000	4	4
A A	68.422500	4	6
A A	68.073125	16	1
A A	66.570000	22	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=221.926

GROUPING	MEAN	N	DIET
A	73.498500	20	3
A A	69.503333	30	2
A A	69.269500	20	1

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=221.926

GROUPING	MEAN	N	OCA
A	74.404375	16	2
A A	69.444259	54	1

Appendix Table Baiv. Multivariate analysis of variance of the effects of diet and OCA on fat intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 6

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: FAT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	2187.13195401	729.04398467	1.40	0.2512	0.059632	30.7355
ERROR	66	34489.80930457	522.57286825		STD DEV		FAT MEAN
CORRECTED TOTAL	69	36676.94125857			22.85985276		74.37614286

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	30.87911690	0.03	0.9709	2	11.99665400	0.01	0.9886
OCA	1	2156.25283710	4.13	0.0463	1	2156.25283710	4.13	0.0463

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE FAT

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=522.573

GROUPING	MEAN	N	GROUP
A	94.681250	8	5
B A	78.267500	4	6
B A	74.021875	16	1
B A	73.501250	16	3
B A	70.837500	4	4
B	67.822273	22	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=522.573

GROUPING	MEAN	N	DIET
A	74.984667	30	2
A A	74.454500	20	3
A	73.385000	20	1

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=522.573

GROUPING	MEAN	N	OCA
A	84.616875	16	2
B	71.341852	54	1

Appendix Table Bav. Multivariate analysis of variance of the effects of diet and OCA on calcium intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 7

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: CALCIUM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	187274.19434472	62424.73144824	0.71	0.5548	0.031128	36.6350
ERROR	66	5829004.73136952	88318.25350560		STD DEV		CALCIUM MEAN
CORRECTED TOTAL	69	6016278.92571424			297.18387154		811.11428571

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	133318.36454762	0.75	0.4741	2	145708.06800543	0.82	0.4427
OCA	1	53955.82979710	0.61	0.4372	1	53955.82979710	0.61	0.4372

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE CALCIUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=88318.3

GROUPING	MEAN	N	GROUP
A	867.031250	16	3
A A	861.212500	8	5
A A	855.750000	4	4
A A	845.350000	4	6
A A	826.675000	16	1
A A	726.572727	22	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=88318.3

GROUPING	MEAN	N	DIET
A	862.695000	20	3
A A	832.490000	20	1
A A	762.476667	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=88318.3

GROUPING	MEAN	N	OCA
A	855.881250	16	2
A A	797.850000	54	1

Appendix Table Bavi. Multivariate analysis of variance of the effects of diet and OCA on iron intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 8

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: IRON

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	5.64361522	1.88120507	0.45	0.7219	0.020037	18.8339
ERROR	66	276.00990478	4.18196825		STD DEV		IRON MEAN
CORRECTED TOTAL	69	281.65352000			2.04498613		10.85600000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	3.11138333	0.37	0.6908	2	3.54292948	0.42	0.6565
OCA	1	2.53223188	0.61	0.4393	1	2.53223188	0.61	0.4393

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE IRON

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=4.18197

GROUPING	MEAN	N	GROUP
A	11.433750	8	5
A A	11.093125	16	3
A A	11.043750	16	1
A A	11.030000	4	6
A A	10.807500	4	4
A	10.320455	22	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=4.18197

GROUPING	MEAN	N	DIET
A	11.080500	20	3
A A	10.996500	20	1
A	10.617333	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=4.18197

GROUPING	MEAN	N	OCA
A	11.176250	16	2
A	10.763704	54	1

Appendix Table Bavii. Multivariate analysis of variance of the effects of diet and OCA on vitamin A intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 9

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: VITA

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	62166811.47687876	20722270.49229292	4.73	0.0049	0.176836	39.1594
ERROR	66	289384552.01298057	4384614.42443910		STD DEV		VITA MEAN
CORRECTED TOTAL	69	351551363.48985933			2093.94709208		5347.24142857

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	27680982.42035687	3.16	0.0491	2	23614939.37139643	2.69	0.0751
OCA	1	34485829.05652189	7.87	0.0066	1	34485829.05652188	7.87	0.0066

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE VITA

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=4384614

GROUPING	MEAN	N	GROUP
A	6727.025000	16	3
A			
B A	5934.743750	16	1
B			
B C	4908.022727	22	2
B C			
D C	4300.500000	4	6
B C			
B C	4218.537500	8	5
C	3197.950000	4	4

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=4384614

GROUPING	MEAN	N	DIET
A	6241.720000	20	3
A			
B A	5387.385000	20	1
B			
B	4724.160000	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=4384614

GROUPING	MEAN	N	OCA
A	5751.200000	54	1
B	3983.881250	16	2

Appendix Table Baviii. Multivariate analysis of variance of the effects of diet and OCA on thiamine intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 10

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: THIAM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	0.12033033	0.04011011	0.42	0.7404	0.018873	28.6388
ERROR	66	6.25561967	0.09478212		STD DEV		THIAM MEAN
CORRECTED TOTAL	69	6.37595000			0.30786704		1.07500000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	0.11906333	0.63	0.5368	2	0.12025741	0.63	0.5335
OCA	1	0.00126699	0.01	0.9083	1	0.00126699	0.01	0.9083

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE THIAM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=.0947821

GROUPING	MEAN	N	GROUP
A	1.162500	4	6
A	1.135625	16	1
A	1.105000	16	3
A	1.082500	8	5
A	1.007727	22	2
A	0.980000	4	4

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=.0947821

GROUPING	MEAN	N	DIET
A	1.116500	20	3
A	1.104500	20	1
A	1.027667	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=.0947821

GROUPING	MEAN	N	OCA
A	1.076875	16	2
A	1.074444	54	1

Appendix Table B.ix. Multivariate analysis of variance of the effects of diet and OCA on riboflavin intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 11

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RIBO

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	0.27594640	0.09198213	0.52	0.6754	0.022999	27.6509
ERROR	66	11.72235217	0.17761140		STD DEV		RIBO MEAN
CORRECTED TOTAL	69	11.99829857			0.42143967		1.52414286

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	0.24352190	0.69	0.5074	2	0.25456542	0.72	0.4922
OCA	1	0.03242449	0.18	0.6706	1	0.03242449	0.18	0.6706

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RIBO

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=0.177611

GROUPING	MEAN	N	GROUP
A	1.608125	16	3
A A	1.607500	4	6
A A	1.576250	8	5
A A	1.543750	16	1
A A	1.465000	4	4
A A	1.425455	22	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=0.177611

GROUPING	MEAN	N	DIET
A	1.608000	20	3
A A	1.528000	20	1
A	1.465667	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=0.177611

GROUPING	MEAN	N	OCA
A	1.556250	16	2
A	1.514630	54	1

Appendix Table Bax. Multivariate analysis of variance of the effects of diet and OCA on vitamin C intake

S T A T I S T I C A L A N A L Y S I S S Y S T E M 12

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: VITC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	8108.97470031	2702.99156677	2.16	0.0956	0.089407	46.9920
ERROR	66	82588.20872826	1251.33649588		STD DEV		VITC MEAN
CORRECTED TOTAL	69	90697.18342857			35.37423452		75.27714286

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	7948.31426190	3.18	0.0482	2	7778.83914211	3.11	0.0513
OCA	1	160.66043841	0.13	0.7212	1	160.66043841	0.13	0.7212

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE VITC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=1251.34

GROUPING	MEAN	N	GROUP
A	93.806250	16	3
A			
B A	80.825000	4	6
B A			
B A	80.568750	16	1
B A			
B A	78.487500	8	5
B			
B	60.854545	22	2
B			
B	47.350000	4	4

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=1251.34

GROUPING	MEAN	N	DIET
A	91.210000	20	3
A			
B A	73.925000	20	1
B			
B	65.556667	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=1251.34

GROUPING	MEAN	N	OCA
A	76.459259	54	1
A			
A	71.287500	16	2

Appendix Table Baxi. Multivariate analysis of variance of the effects of diet and OCA on total folate intake

STATISTICAL ANALYSIS SYSTEM 13

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: TFOLATE

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	3	6056.18460248	2018.72820083	1.18	0.3250	0.050812	25.9756
ERROR	66	113131.01382609	1714.10627009		STD DEV		TFOLATE MEAN
CORRECTED TOTAL	69	119187.19842857			41.40176651		159.38714286

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	4189.84976190	1.22	0.3012	2	3753.31154428	1.09	0.3406
OCA	1	1866.33484058	1.09	0.3005	1	1866.33484058	1.09	0.3005

BIOAVAILABILITY OF FA FROM OJUICE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE TFOLATE

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=1714.11

GROUPING	MEAN	N	GROUP
A	170.800000	16	3
A A	166.656250	16	1
A A	159.075000	4	6
A A	153.463636	22	2
A A	149.800000	4	4
A A	143.262500	8	5

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=1714.11

GROUPING	MEAN	N	DIET
A	168.455000	20	3
A A	163.285000	20	1
A A	150.743333	30	2

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=66 MS=1714.11

GROUPING	MEAN	N	OCA
A	162.509259	54	1
A A	148.850000	16	2

Appendix Table Baxii. Mean daily nutrient intake by food groups

Food group	Consumption (g)	Energy (kcal)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)	Vitamin A (IU)	Thiamine (mg)	Riboflavin (mg)	Folate (ug)	Vitamin C (mg)
	Nutrient intake/person/day										
Dairy products	304	286	18	15	551	0.4	578	0.12	0.67	21	3
Meat, poultry, fish, eggs	165	362	29	26	29	3.4	216	0.32	0.29	16	tr
Cereal products	159	489	12	13	98	2.9	150	0.33	0.24	40	1
Fruit and fruit products[a]	227	143	1	tr	23	1.1	332	0.07	0.06	19	21
Vegetables and potatoes	191	82	3	1	46	1.4	3035	0.13	0.11	41	45
Nuts and legumes	23	32	2	2	10	0.5	350	0.05	0.03	10	2
Cooking oils and fats	20	117	1	12	6	0.1	271	0.01	0.01	2	tr
Sugars and adjuncts	346	98	1	1	13	0.4	24	0.01	0.04	1	tr
Mixed dishes/ soups	80	90	4	4	35	0.8	479	0.04	0.07	9	3
Total	1515	1699	71	74	811	11.0	5347	1.08	1.52	159	75

[a]Excludes orange juice supplementation
tr = negligible quantity present

Appendix Table Baxiii. Percent contribution to nutrient intakes by food groups

Food group	Percent contribution									
	Energy (kcal)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)	Vitamin A (IU)	Thiamine (mg)	Riboflavin (mg)	Folate (ug)	Vitamin C (mg)
Dairy products	17	25	20	67	3	11	11	44	13	4
Meat, poultry, fish, eggs	21	42	35	4	31	4	29	19	10	0
Cereal products	28	17	17	12	26	3	30	15	25	1
Fruit and fruit products[a]	8	2	1	3	10	6	6	4	12	28
Vegetables and potatoes	5	4	2	6	14	56	13	8	27	59
Nuts and legumes	2	2	2	1	5	6	5	1	6	3
Cooking oils and fats	7	1	16	1	1	5	1	1	1	1
Sugars and adjuncts	6	1	2	2	3	0	1	3	1	1
Mixed dishes/soups	6	6	5	4	7	9	4	5	5	3

[a]Excludes orange juice supplementation

b. Clinical data

Appendix Table Bbi. Serum and erythrocyte folates of individual subjects

STATISTICAL ANALYSIS SYSTEM 1

OBS	SUBJECT	T	DIET	OCA	SERUM	RBC	N	SAVES	SAVE2	LAST	FSERUM	FRBC
1	1	0	1	1	11.6	437	2	9.2	271	1	1.26047	1.61255
2	2	0	1	1	16.0	319	7	9.2	591	2	1.73913	0.53976
3	3	0	1	1	18.0	328	12	12.8	349	3	1.65667	0.93983
4	4	0	1	1	13.0	400	17	8.0	378	4	1.46067	1.05820
5	5	0	1	1	8.7	431	22	6.8	203	5	1.27941	2.12315
6	6	0	1	1	11.8	259	27	9.4	316	6	1.28536	0.81962
7	7	0	1	1	12.6	295	32	12.2	365	7	1.03279	0.78082
8	8	0	1	1	10.8	198	37	5.7	257	8	1.89474	0.77049
9	9	0	1	1	9.9	738	42	7.2	247	9	1.37500	2.98785
10	10	0	1	1	9.7	464	47	6.8	194	10	1.42647	2.39485
11	11	0	1	1	9.6	520	52	7.5	247	11	1.28267	2.10526
12	12	0	1	1	8.0	495	57	6.5	346	12	1.23077	1.43064
13	40	0	1	2	9.3	457	195	9.3	369	40	1.00376	1.23848
14	41	0	1	2	11.2	296	200	6.1	311	41	1.83607	0.95177
15	42	0	1	2	5.8	515	205	5.3	295	42	1.09434	1.80702
16	43	0	1	2	8.1	575	210	5.4	301	43	1.50562	1.91070
17	44	0	1	2	6.5	543	215	4.6	250	44	1.41304	2.17200
18	45	0	1	2	6.2	568	220	5.6	311	45	1.10714	1.82637
19	46	0	1	2	6.9	390	225	6.6	319	46	1.04545	1.22257
20	13	0	2	1	13.3	622	62	7.4	269	13	1.79730	2.34717
21	14	0	2	1	12.3	293	67	13.9	354	14	0.88136	0.82768
22	15	0	2	1	10.5	261	72	9.6	200	15	1.09375	1.30500
23	16	0	2	1	40.0	363	77	23.0	399	16	2.17043	0.93377
24	17	0	2	1	36.0	425	82	11.1	393	17	3.15315	1.08142
25	18	0	2	1	15.0	443	87	8.5	338	18	1.76471	1.14175
26	19	0	2	1	6.9	407	92	6.9	327	19	1.00000	1.24465
27	20	0	2	1	13.4	391	97	9.4	431	20	1.42553	0.90719
28	21	0	2	1	12.7	187	100	13.2	222	21	0.96212	0.84234
29	22	0	2	1	8.0	451	105	6.6	248	22	1.21212	1.81855
30	23	0	2	1	20.0	764	110	9.2	333	23	2.17391	2.29426
31	24	0	2	1	6.3	355	115	6.3	239	24	1.00000	1.48536
32	25	0	2	1	7.0	452	120	6.0	204	25	1.16667	2.21569
33	26	0	2	1	15.0	657	125	8.3	308	26	1.80723	2.13312
34	47	0	2	2	18.0	497	230	10.9	385	47	1.65175	1.29091
35	48	0	2	2	7.6	602	235	5.4	329	48	1.40741	1.82979
36	49	0	2	2	18.0	408	240	15.0	443	49	1.20000	0.92099
37	50	0	2	2	9.7	440	245	5.0	196	50	1.94000	2.24490
38	51	0	2	2	4.0	534	250	3.7	138	51	1.08158	2.69807
39	52	0	2	2	10.6	492	255	12.0	286	52	0.88333	1.72028
40	53	0	2	2	6.9	498	260	5.2	196	53	1.13462	2.54082
41	27	0	3	1	16.0	325	140	15.0	195	27	1.06667	1.21026
42	28	0	3	1	12.1	419	135	16.0	283	28	0.90067	1.46067
43	29	0	3	1	16.0	443	140	10.1	412	29	1.58416	1.07524
44	30	0	3	1	80.0	612	145	11.9	632	30	6.72269	0.96835
45	31	0	3	1	12.2	463	150	6.6	283	31	1.76812	1.63604
46	32	0	3	1	13.1	373	155	7.0	398	32	1.87143	0.93719
47	33	0	3	1	80.0	310	160	13.9	372	33	5.75540	0.83333
48	34	0	3	1	6.4	470	165	5.0	196	34	1.28000	2.44388
49	35	0	3	1	9.3	467	170	5.9	284	35	1.57627	1.64437
50	36	0	3	1	11.0	571	175	7.8	255	36	1.41026	2.23922
51	37	0	3	1	7.8	531	180	7.3	220	37	1.06849	2.41364
52	38	0	3	1	8.1	477	185	5.5	240	38	1.47273	1.98750
53	39	0	3	1	7.2	523	190	6.4	204	39	1.12500	2.56373
54	54	0	3	2	10.6	305	262	10.7	252	54	0.98131	1.21032

continued

B18 contd.

Table Bbi (continued)

STATISTICAL ANALYSIS SYSTEM 2

OBS	SUBJECT	T	DIET	OCA	SERUM	RBC	N	SAVES	SAVER	LAST	FSERUM	FRBC
55	55	0	3	2	7.4	220	267	6.0	195	55	1.23333	1.12821
56	56	0	3	2	7.2	558	272	6.1	213	56	1.18033	2.61972
57	57	0	3	2	7.6	593	275	6.6	239	57	1.15152	2.48117
58	58	0	3	2	7.7	469	280	9.1	280	58	0.84615	1.67500
59	59	0	3	2	4.8	526	285	4.1	269	59	1.17073	1.95630
60	60	0	3	2	10.4	616	290	7.9	262	60	1.32051	2.10440
61	1	2	1	1	9.2	271	1	9.2	271	1	1.00000	1.00000
62	2	2	1	1	9.2	591	6	9.2	591	2	1.00000	1.00000
63	3	2	1	1	10.8	349	11	10.8	349	3	1.00000	1.00000
64	4	2	1	1	8.9	378	16	8.9	378	4	1.00000	1.00000
65	5	2	1	1	6.8	203	21	6.8	203	5	1.00000	1.00000
66	6	2	1	1	9.4	316	26	9.4	316	6	1.00000	1.00000
67	7	2	1	1	12.2	365	31	12.2	365	7	1.00000	1.00000
68	8	2	1	1	5.7	257	36	5.7	257	8	1.00000	1.00000
69	9	2	1	1	7.2	247	41	7.2	247	9	1.00000	1.00000
70	10	2	1	1	6.8	194	46	6.8	194	10	1.00000	1.00000
71	11	2	1	1	7.5	247	51	7.5	247	11	1.00000	1.00000
72	12	2	1	1	6.5	346	56	6.5	346	12	1.00000	1.00000
73	40	2	1	2	9.3	369	194	9.3	369	40	1.00000	1.00000
74	41	2	1	2	6.1	311	199	6.1	311	41	1.00000	1.00000
75	42	2	1	2	5.3	265	204	5.3	265	42	1.00000	1.00000
76	43	2	1	2	6.4	301	209	6.4	301	43	1.00000	1.00000
77	44	2	1	2	4.6	250	214	4.6	250	44	1.00000	1.00000
78	45	2	1	2	5.6	311	219	5.6	311	45	1.00000	1.00000
79	46	2	1	2	6.6	319	224	6.6	319	46	1.00000	1.00000
80	13	2	2	1	7.4	265	61	7.4	265	13	1.00000	1.00000
81	14	2	2	1	13.9	354	66	13.9	354	14	1.00000	1.00000
82	15	2	2	1	9.6	200	71	9.6	200	15	1.00000	1.00000
83	16	2	2	1	23.0	390	76	23.0	390	16	1.00000	1.00000
84	17	2	2	1	11.1	393	81	11.1	393	17	1.00000	1.00000
85	18	2	2	1	8.5	388	86	8.5	388	18	1.00000	1.00000
86	19	2	2	1	6.9	327	91	6.9	327	19	1.00000	1.00000
87	20	2	2	1	9.4	431	96	9.4	431	20	1.00000	1.00000
88	21	2	2	1	13.2	222	99	13.2	222	21	1.00000	1.00000
89	22	2	2	1	6.6	248	104	6.6	248	22	1.00000	1.00000
90	23	2	2	1	9.2	333	109	9.2	333	23	1.00000	1.00000
91	24	2	2	1	6.3	239	114	6.3	239	24	1.00000	1.00000
92	25	2	2	1	6.0	204	119	6.0	204	25	1.00000	1.00000
93	26	2	2	1	8.3	308	124	8.3	308	26	1.00000	1.00000
94	47	2	2	2	10.9	385	230	10.9	385	47	1.00000	1.00000
95	48	2	2	2	5.4	329	234	5.4	329	48	1.00000	1.00000
96	49	2	2	2	15.0	443	239	15.0	443	49	1.00000	1.00000
97	50	2	2	2	6.0	196	244	5.0	196	50	1.00000	1.00000
98	51	2	2	2	3.7	198	249	3.7	198	51	1.00000	1.00000
99	52	2	2	2	12.0	286	254	12.0	286	52	1.00000	1.00000
100	53	2	2	2	5.2	196	259	5.2	196	53	1.00000	1.00000
101	27	2	3	1	15.0	185	129	15.0	185	27	1.00000	1.00000
102	28	2	3	1	15.0	283	134	15.0	283	28	1.00000	1.00000
103	29	2	3	1	10.1	412	139	10.1	412	29	1.00000	1.00000
104	30	2	3	1	11.9	632	144	11.9	632	30	1.00000	1.00000
105	31	2	3	1	6.9	283	149	6.9	283	31	1.00000	1.00000
106	32	2	3	1	7.0	398	154	7.0	398	32	1.00000	1.00000
107	33	2	3	1	13.9	372	159	13.9	372	33	1.00000	1.00000
108	34	2	3	1	5.0	196	164	5.0	196	34	1.00000	1.00000

continued

B18 contd.

Appendix Table Bb1 (continued)

STATISTICAL ANALYSIS SYSTEM 3

OBS	SUBJECT	T	DIET	OCA	SERUM	RBC	N	SAVES	SAVER	LAST	RSERUM	RRBC
109	35	2	3	1	5.9	284	169	5.9	284	35	1.00000	1.00000
110	36	2	3	1	7.8	255	174	7.8	255	36	1.00000	1.00000
111	37	2	3	1	7.7	220	179	7.7	220	37	1.00000	1.00000
112	38	2	3	1	5.5	240	184	5.5	240	38	1.00000	1.00000
113	39	2	3	1	6.4	204	189	6.4	204	39	1.00000	1.00000
114	54	2	3	2	10.7	252	261	10.7	252	54	1.00000	1.00000
115	55	2	3	2	6.0	195	266	6.0	195	55	1.00000	1.00000
116	56	2	3	2	6.1	213	271	6.1	213	56	1.00000	1.00000
117	57	2	3	2	6.6	239	274	6.6	239	57	1.00000	1.00000
118	58	2	3	2	9.1	280	279	9.1	280	58	1.00000	1.00000
119	59	2	3	2	4.1	269	284	4.1	269	59	1.00000	1.00000
120	60	2	3	2	7.8	282	289	7.8	282	60	1.00000	1.00000
121	1	5	1	1	6.9	470	3	9.2	271	1	0.75000	1.73432
122	2	5	1	1	91.0	327	8	9.2	501	2	9.89130	0.65330
123	3	5	1	1	29.0	363	13	10.8	349	3	2.68519	1.04011
124	4	5	1	1	13.0	222	18	8.9	378	4	1.46067	0.58730
125	5	5	1	1	9.3	173	23	6.9	203	5	1.45294	0.85222
126	6	5	1	1	5.9	302	28	9.4	316	6	0.62766	0.95570
127	7	5	1	1	9.0	374	33	12.2	365	7	0.73770	0.81507
128	8	5	1	1	8.5	325	38	5.7	257	8	1.49123	1.26459
129	9	5	1	1	13.5	285	43	7.9	247	9	1.70411	1.15385
130	10	5	1	1	9.4	175	48	6.8	174	10	1.38235	0.90206
131	11	5	1	1	10.2	199	53	7.5	247	11	1.36000	0.80567
132	12	5	1	1	6.5	142	58	6.5	346	12	1.00000	0.41040
133	40	5	1	2	10.2	341	196	9.3	369	40	1.09677	0.92412
134	41	5	1	2	13.0	339	201	6.1	311	41	2.13115	1.08682
135	42	5	1	2	7.9	264	206	5.3	285	42	1.49057	0.92632
136	43	5	1	2	5.9	266	211	6.4	301	43	0.92188	0.88037
137	44	5	1	2	9.2	218	216	4.6	250	44	2.00000	0.87200
138	45	5	1	2	9.1	236	221	5.6	311	45	1.62500	0.75884
139	46	5	1	2	9.6	182	226	6.6	319	46	1.45455	0.57053
140	13	5	2	1	11.4	.	63	7.4	265	13	1.54054	.
141	14	5	2	1	10.6	470	68	13.2	354	14	0.74242	1.32768
142	15	5	2	1	9.9	377	73	9.6	200	15	1.03125	1.88500
143	16	5	2	1	68.0	505	78	23.0	300	16	2.73913	1.26667
144	17	5	2	1	12.4	232	83	11.1	393	17	1.11712	0.59033
145	18	5	2	1	9.1	353	88	8.5	354	18	0.95294	0.99379
146	19	5	2	1	43.0	361	93	6.9	327	19	6.23188	1.10514
147	20	5	2	1	10.8	403	98	9.4	431	20	1.14894	0.93503
148	21	5	2	1	11.0	198	101	13.2	222	21	0.83333	0.89189
149	22	5	2	1	10.3	207	106	6.6	248	22	1.56152	0.83468
150	23	5	2	1	19.0	301	111	9.2	333	23	2.06522	0.90390
151	24	5	2	1	9.0	200	116	6.3	239	24	1.42857	0.83682
152	25	5	2	1	9.5	182	121	6.0	204	25	1.58333	0.89216
153	26	5	2	1	11.7	269	126	9.3	308	26	1.25806	0.87013
154	47	5	2	2	20.0	274	231	10.0	385	47	1.93456	0.71169
155	48	5	2	2	10.6	287	236	9.4	329	48	1.06298	0.87234
156	49	5	2	2	13.6	524	241	15.0	443	49	0.90667	1.18284
157	50	5	2	2	9.0	109	246	5.0	196	50	1.80000	0.55612
158	51	5	2	2	8.8	218	251	3.7	198	51	2.37838	1.10101
159	52	5	2	2	32.0	319	256	12.0	286	52	2.66667	1.11538
160	27	5	3	1	72.0	390	131	15.0	195	27	4.80000	2.10811
161	28	5	3	1	22.0	363	136	15.0	283	28	1.46667	1.22269
162	29	5	3	1	74.0	434	141	10.1	412	29	7.32673	1.05340

B18 contd.

continued

Appendix Table Bbi (continued)

STATISTICAL ANALYSIS SYSTEM

OBS	SUBJECT	T	DIET	OCA	SERUM	RBC	N	SAVES	SAVER	LAST	FSERUM	FRBC
163	30	5	3	1	22.0	305	146	11.9	632	30	1.5687	0.79905
164	31	5	3	1	15.0	381	151	6.9	243	31	2.1739	1.34629
165	32	5	3	1	86.0	194	156	7.0	398	32	12.2857	0.46744
166	33	5	3	1	26.0	416	161	13.9	372	33	2.5869	1.11028
167	34	5	3	1	12.4	170	166	5.0	196	34	2.4800	0.85735
168	35	5	3	1	23.0	239	171	5.9	284	35	3.7200	0.84155
169	36	5	3	1	29.0	168	176	7.8	255	36	3.7179	0.65962
170	37	5	3	1	11.1	287	181	7.3	220	37	1.5205	1.30455
171	38	5	3	1	10.6	198	186	5.5	240	38	1.9273	0.82500
172	39	5	3	1	8.9	192	191	6.4	204	39	1.3906	0.94118
173	54	5	3	2	15.0	345	267	10.7	252	54	1.4019	1.36905
174	55	5	3	2	7.6	131	268	6.0	195	55	1.2667	0.67179
175	56	5	3	2	9.1	209	273	6.1	213	56	1.4918	0.98122
176	57	5	3	2	12.4	338	276	6.6	239	57	1.8788	1.41423
177	58	5	3	2	19.0	310	281	9.1	230	58	1.9740	1.16714
178	59	5	3	2	8.2	317	286	4.1	269	59	2.0000	1.17944
179	60	5	3	2	10.0	261	291	7.8	232	60	1.2821	0.92553
180	1	9	1	1	7.8	264	4	9.2	271	1	0.8478	0.97417
181	2	9	1	1	10.3	145	9	9.2	591	2	1.1196	0.24535
182	3	9	1	1	12.4	197	14	10.8	349	3	1.1481	0.56447
183	4	9	1	1	9.2	342	19	8.9	378	4	1.0337	0.90476
184	5	9	1	1	11.3	171	24	6.8	203	5	1.6618	0.84236
185	6	9	1	1	6.9	230	29	9.4	316	6	0.7334	0.72785
186	7	9	1	1	8.6	309	34	12.2	365	7	0.7049	0.84384
187	8	9	1	1	4.8	197	39	5.7	257	8	0.8421	0.76654
188	9	9	1	1	8.4	388	44	7.2	247	9	1.1967	1.57085
189	10	9	1	1	8.0	289	49	6.8	194	10	1.1765	1.48969
190	11	9	1	1	9.3	236	54	7.5	247	11	1.2400	0.95547
191	12	9	1	1	7.0	264	59	6.5	346	12	1.2154	0.76301
192	40	9	1	2	4.7	188	197	9.3	368	40	0.5054	0.50949
193	41	9	1	2	6.7	185	202	6.1	311	41	1.0984	0.59486
194	42	9	1	2	9.4	200	207	5.3	295	42	1.7736	0.70175
195	43	9	1	2	8.8	192	212	6.4	301	43	1.3750	0.63747
196	44	9	1	2	6.5	221	217	4.6	250	44	1.4130	0.88400
197	45	9	1	2	7.6	300	222	5.6	311	45	1.3571	0.96463
198	46	9	1	2	9.1	325	227	6.6	319	46	1.3788	1.01881
199	13	9	2	1	15.0	470	64	7.4	205	13	2.3077	1.77354
200	14	9	2	1	12.5	214	69	13.9	354	14	0.8993	0.60452
201	15	9	2	1	12.8	329	74	9.6	200	15	1.3333	1.64500
202	16	9	2	1	11.1	313	79	23.0	390	16	0.4826	0.80256
203	17	9	2	1	12.8	454	84	11.1	393	17	1.0941	1.15522
204	18	9	2	1	15.0	505	89	8.5	348	18	1.7647	1.30155
205	19	9	2	1	13.0	319	94	6.9	327	19	2.0145	0.97554
206	21	9	2	1	20.0	327	102	13.2	222	21	1.5152	1.47297
207	22	9	2	1	10.2	297	107	6.6	248	22	1.5455	1.19758
208	23	9	2	1	12.3	250	112	9.2	333	23	1.3261	0.75075
209	24	9	2	1	9.5	174	117	6.3	239	24	1.5079	0.72803
210	25	9	2	1	11.2	233	122	6.0	204	25	1.8667	1.14216
211	26	9	2	1	15.0	327	127	8.3	308	26	1.8072	1.06169
212	47	9	2	2	29.0	345	232	10.9	335	47	2.6606	0.99610
213	48	9	2	2	16.0	550	237	5.4	329	48	2.9630	1.67173
214	49	9	2	2	30.0	310	242	15.0	443	49	2.0300	0.69977
215	50	9	2	2	9.2	200	247	5.0	196	50	1.8400	1.02041
216	51	9	2	2	6.5	275	252	3.7	198	51	1.7568	1.38889

B18 contd.

continued

Appendix Table Bbi (continued)

STATISTICAL ANALYSIS SYSTEM 5

OBS	SUBJECT	T	DIET	OCA	SERUM	RBC	N	SAVES	SAVER	LAST	PSERUM	PERC
217	52	9	2	2	15.0	257	257	12.0	286	52	1.25000	0.89850
218	27	9	3	1	8.9	174	132	15.0	185	27	0.59333	0.94054
219	28	9	3	1	100.0	257	137	15.0	283	28	6.66667	0.90813
220	29	9	3	1	16.0	434	142	10.1	412	29	1.58416	1.05240
221	30	9	3	1	79.0	532	147	11.9	632	30	6.63866	0.84177
222	31	9	3	1	11.7	321	152	6.9	283	31	1.69565	1.13428
223	32	9	3	1	15.0	443	157	7.0	398	32	2.14286	1.11307
224	33	9	3	1	30.0	239	162	13.9	372	33	2.15827	0.64247
225	34	9	3	1	8.9	269	167	5.0	196	34	1.78000	1.37184
226	35	9	3	1	10.5	367	172	5.9	284	35	1.77966	1.29225
227	36	9	3	1	9.2	271	177	7.3	255	36	1.17749	1.06275
228	37	9	3	1	12.9	329	182	7.3	220	37	1.76712	1.49545
229	38	9	3	1	9.4	173	187	5.5	240	38	1.70909	0.72083
230	39	9	3	1	15.0	265	192	6.4	204	39	2.34375	1.29902
231	54	9	3	2	16.0	239	264	10.7	252	54	1.49533	0.94841
232	55	9	3	2	9.7	360	269	6.0	195	55	1.61667	1.84615
233	57	9	3	2	11.1	313	277	6.6	239	57	1.68182	1.30962
234	58	9	3	2	9.2	227	282	9.1	280	58	1.01099	0.81071
235	59	9	3	2	6.7	283	287	4.1	269	59	1.63415	1.05204
236	60	9	3	2	10.6	207	292	7.8	282	60	1.35897	0.73404
237	1	11	1	1	8.1	157	5	9.2	271	1	0.88043	0.57934
238	2	11	1	1	9.7	191	10	9.2	591	2	1.05435	0.32318
239	3	11	1	1	12.5	392	15	10.8	349	3	1.15741	1.12321
240	4	11	1	1	9.2	431	20	8.9	376	4	1.03371	1.14721
241	5	11	1	1	12.8	419	25	6.3	203	5	1.48335	2.05911
242	6	11	1	1	10.4	243	30	9.4	316	6	1.10328	0.76239
243	7	11	1	1	12.3	223	35	12.2	365	7	1.00820	0.61096
244	8	11	1	1	9.8	209	40	5.7	257	8	1.71930	0.80934
245	9	11	1	1	10.5	304	45	7.2	247	9	1.45833	1.50614
246	10	11	1	1	10.4	208	50	6.3	194	10	1.65741	1.07216
247	11	11	1	1	9.0	209	55	7.5	247	11	1.20000	0.84615
248	12	11	1	1	10.0	252	60	6.5	346	12	1.53847	0.72832
249	40	11	1	2	10.0	235	194	9.3	369	40	1.07527	0.63689
250	41	11	1	2	9.9	386	203	6.1	311	41	1.62295	1.24116
251	42	11	1	2	5.0	285	208	5.3	285	42	0.94440	1.00000
252	43	11	1	2	10.4	296	213	6.4	401	43	1.62500	0.68339
253	44	11	1	2	5.4	223	218	4.5	250	44	1.17331	0.89200
254	45	11	1	2	6.3	177	223	5.6	311	45	1.12500	0.56913
255	46	11	1	2	9.0	217	228	6.5	319	46	1.50000	0.68025
256	13	11	2	1	11.9	295	65	7.4	265	13	1.60811	1.11321
257	14	11	2	1	11.7	224	70	13.9	354	14	0.84173	0.63277
258	15	11	2	1	10.1	181	75	9.6	200	15	1.05208	0.90500
259	16	11	2	1	12.0	303	80	23.0	393	16	0.52174	0.77099
260	17	11	2	1	15.5	514	85	11.1	393	17	1.39640	1.30789
261	18	11	2	1	12.5	401	90	9.5	348	18	1.47368	1.03351
262	19	11	2	1	13.8	393	95	6.9	327	19	2.00000	1.19266
263	21	11	2	1	13.4	186	103	13.2	222	21	1.01515	0.83784
264	22	11	2	1	9.6	200	109	5.6	248	22	1.45455	0.80645
265	23	11	2	1	17.0	167	113	9.2	333	23	1.84783	0.50150
266	24	11	2	1	12.0	197	118	6.3	239	24	1.90476	0.82427
267	25	11	2	1	8.8	236	123	6.0	204	25	1.46667	1.15686
268	26	11	2	1	11.0	225	128	8.3	308	26	1.32530	0.73052
269	47	11	2	2	12.0	269	233	10.9	385	47	1.10092	0.69810
270	48	11	2	2	15.6	154	238	5.4	329	48	1.98296	0.46809

continued

B18 contd.

Appendix Table Bbi (continued)

STATISTICAL ANALYSIS SYSTEM 6

OBS	SUBJECT	T	DIFT	OCA	SERUM	FBC	N	SAVES	SAVEF	LAST	FSERUM	FFBC
271	49	11	2	2	95.0	425	243	15.0	443	49	6.33333	6.95937
272	50	11	2	2	6.7	194	248	5.0	196	50	1.44000	0.68080
273	51	11	2	2	7.2	255	253	4.7	196	51	1.44595	1.33838
274	52	11	2	2	33.0	292	288	12.0	246	52	2.75000	1.22098
275	27	11	3	1	92.0	336	133	15.0	185	27	6.13333	1.91692
276	28	11	3	1	37.0	185	138	15.0	283	28	2.46667	0.65371
277	29	11	3	1	13.1	560	143	10.1	412	29	1.29703	1.35932
278	30	11	3	1	15.0	612	148	11.0	632	30	1.36050	0.96835
279	31	11	3	1	12.0	472	153	6.0	283	31	1.73813	1.66724
280	32	11	3	1	33.0	514	158	7.0	398	32	4.71429	1.29146
281	33	11	3	1	95.0	407	163	13.0	372	33	6.83453	1.09409
282	34	11	3	1	9.7	235	168	5.0	196	34	1.94000	1.19808
283	35	11	3	1	13.8	257	173	5.9	284	35	2.33898	0.90483
284	36	11	3	1	10.8	314	178	7.8	255	36	1.38462	1.23137
285	37	11	3	1	11.6	.	183	7.3	220	37	1.58904	.
286	38	11	3	1	20.0	203	188	5.5	243	38	3.63636	0.84583
287	39	11	3	1	11.6	268	193	6.4	204	39	1.81250	1.31373
288	54	11	3	2	11.8	259	265	10.7	252	54	1.10280	1.02778
289	55	11	3	2	11.4	517	270	6.0	195	55	1.90000	2.65128
290	57	11	3	2	11.6	296	278	6.6	233	57	1.75758	1.19665
291	58	11	3	2	12.7	151	283	9.1	280	58	1.39560	0.53320
292	59	11	3	2	9.2	351	288	4.1	269	59	2.24390	1.30483
293	60	11	3	2	16.0	301	293	7.8	282	60	2.05128	1.06738

Appendix Table Bbii. Mean serum and erythrocyte folates and their calculated ratios at blood sampling weeks

T	Diet	OCA	n	Folate, ng/ml[a] Serum	Rserum	RBC	RRBC
0	1	1	12	11.6 ± 0.9	1.41 ± 0.07	408 ± 42	1.47 ± 0.23
	1	2	7	7.6 ± 0.8	1.23 ± 0.11	478 ± 39	1.59 ± 0.17
	1	-	19	10.2 ± 0.8	1.34 ± 0.06	434 ± 30	1.52 ± 0.15
0	2	1	14	16.1 ± 3.2	1.54 ± 0.17	433 ± 41	1.47 ± 0.15
	2	2	7	10.5 ± 2.1	1.33 ± 0.14	496 ± 24	1.89 ± 0.25
	2	-	21	14.2 ± 2.3	1.47 ± 0.12	454 ± 29	1.61 ± 0.13
0	3	1	13	21.5 ± 7.3	2.12 ± 0.52	462 ± 24	1.70 ± 0.17
	3	2	7	7.9 ± 0.7	1.13 ± 0.06	470 ± 57	1.89 ± 0.22
	3	-	20	16.7 ± 4.9	1.77 ± 0.35	465 ± 25	1.76 ± 0.13
0	-	1	39	16.5 ± 2.7	1.69 ± 0.19	435 ± 21	1.54 ± 0.10
	-	2	21	8.7 ± 0.8	1.23 ± 0.06	481 ± 23	1.79 ± 0.12
2	1	1	12	8.4 ± 0.6	1.00 ± 0.00	314 ± 31	1.00 ± 0.00
	1	2	7	6.3 ± 0.6	1.00 ± 0.00	307 ± 14	1.00 ± 0.00
	1	-	19	7.6 ± 0.5	1.00 ± 0.00	311 ± 20	1.00 ± 0.00
2	2	1	14	10.0 ± 1.2	1.00 ± 0.00	307 ± 21	1.00 ± 0.00
	2	2	7	8.2 ± 1.7	1.00 ± 0.00	290 ± 38	1.00 ± 0.00
	2	-	21	9.4 ± 1.0	1.00 ± 0.00	302 ± 18	1.00 ± 0.00
2	3	1	13	9.1 ± 1.0	1.00 ± 0.00	305 ± 34	1.00 ± 0.00
	3	2	7	7.2 ± 0.8	1.00 ± 0.00	247 ± 13	1.00 ± 0.00
	3	-	20	8.4 ± 0.7	1.00 ± 0.00	285 ± 23	1.00 ± 0.00
2	-	1	39	9.2 ± 0.6	1.00 ± 0.00	308 ± 16	1.00 ± 0.00
	-	2	21	7.2 ± 0.6	1.00 ± 0.00	281 ± 14	1.00 ± 0.00

[a]Mean ± SEM

continued

Appendix Table Bbii (continued)

T	Diet	OCA	n	Folate, ng/ml[a] Serum	Rserum	RBC	RRBC
5	1	1	12	17.6 ± 6.9	2.04 ± 0.73	276 ± 28	0.93 ± 0.10
	1	2	7	9.1 ± 0.9	1.51 ± 0.18	264 ± 22	0.86 ± 0.06
	1	-	19	14.5 ± 4.4	1.84 ± 0.46	272 ± 19	0.91 ± 0.07
5	2	1	14	17.2 ± 4.2	1.75 ± 0.37	329 ± 39	1.07 ± 0.11
	2	2	6	15.5 ± 3.7	1.89 ± 0.25	289 ± 56	0.92 ± 0.10
	2	-	20	16.7 ± 3.1	1.79 ± 0.27	316 ± 32	1.02 ± 0.08
5	3	1	13	32.4 ± 7.5	3.64 ± 0.86	303 ± 32	1.05 ± 0.11
	3	2	7	11.5 ± 1.5	1.61 ± 0.12	273 ± 30	1.09 ± 0.10
	3	-	20	25.1 ± 5.3	2.93 ± 0.59	292 ± 23	1.06 ± 0.08
5	-	1	39	22.4 ± 3.7	2.47 ± 0.40	303 ± 19	1.02 ± 0.06
	-	2	20	11.9 ± 1.3	1.66 ± 0.11	274 ± 20	0.96 ± 0.05
9	1	1	12	8.8 ± 0.6	1.08 ± 0.08	253 ± 21	0.89 ± 0.10
	1	2	7	7.5 ± 0.6	1.27 ± 0.15	230 ± 22	0.76 ± 0.07
	1	-	19	8.3 ± 0.4	1.15 ± 0.07	244 ± 15	0.84 ± 0.07
9	2	1	13	13.0 ± 0.8	1.46 ± 0.13	324 ± 28	1.12 ± 0.10
	2	2	6	17.6 ± 4.0	2.08 ± 0.26	323 ± 50	1.10 ± 0.15
	2	-	19	14.5 ± 1.4	1.66 ± 0.13	324 ± 24	1.12 ± 0.08
9	3	1	13	25.1 ± 8.2	2.46 ± 0.53	313 ± 30	1.07 ± 0.07
	3	2	6	10.6 ± 1.3	1.47 ± 0.10	272 ± 24	1.12 ± 0.17
	3	-	19	20.5 ± 5.8	2.15 ± 0.38	300 ± 22	1.08 ± 0.07
9	-	1	38	15.8 ± 3.0	1.69 ± 0.21	298 ± 16	1.03 ± 0.05
	-	2	19	11.7 ± 1.6	1.59 ± 0.13	272 ± 20	0.98 ± 0.08

[a]Mean ± SEM

continued

Appendix Table Bbii (continued)

T	Diet	OCA	n	Folate, ng/ml[a] Serum	Rserum	RBC	RRBC
11	1	1	12	10.4 ± 0.4	1.30 ± 0.09	277 ± 29	0.97 ± 0.14
	1	2	7	8.1 ± 0.9	1.30 ± 0.11	260 ± 26	0.86 ± 0.09
	1	-	19	9.6 ± 0.5	1.30 ± 0.07	271 ± 20	0.93 ± 0.09
11	2	1	13	12.3 ± 0.6	1.38 ± 0.12	271 ± 29	0.91 ± 0.07
	2	2	6	27.4 ± 14.1	2.57 ± 0.79	266 ± 38	0.91 ± 0.12
	2	-	19	17.0 ± 4.5	1.75 ± 0.28	269 ± 23	0.91 ± 0.06
11	3	1	13	28.8 ± 8.3	2.86 ± 0.52	364 ± 42	1.20 ± 0.10
	3	2	6	12.1 ± 0.9	1.74 ± 0.17	311 ± 49	1.30 ± 0.29
	3	-	19	23.5 ± 5.9	2.51 ± 0.38	346 ± 32	1.23 ± 0.11
11	-	1	38	17.3 ± 3.1	1.86 ± 0.22	303 ± 20	1.02 ± 0.06
	-	2	19	15.5 ± 4.6	1.84 ± 0.27	278 ± 21	1.01 ± 0.11

[a]Mean ± SEM

Appendix Table Bbiii. Analysis of variance of the effects of diet and OCA on serum folates at week 0

BIOAVAILABILITY OF FA FROM OJUICE
T=0

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SERUM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	1475.18204212	295.03640842	1.53	0.1943	0.124257	100.7307
ERROR	54	10396.83045788	192.53389737		STD DEV		SERUM MEAN
CORRECTED TOTAL	59	11872.01250000			13.87553945		13.77500000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	427.33469424	1.11	0.3370	2	244.83639074	0.64	0.5334
OCA	1	809.24784622	4.20	0.0452	1	805.40744088	4.18	0.0457
DIET*OCA	2	238.60949967	0.62	0.5419	2	238.60949967	0.62	0.5419

BIOAVAILABILITY OF FA FROM OJUICE
T=0

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=192.534

GROUPING	MEAN	N	DIET
A	16.735000	20	3
A	14.223810	21	2
A	10.163158	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=0

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=192.534

GROUPING	MEAN	N	OCA
A	16.505128	39	1
B	8.704762	21	2

Appendix Table Bbiv. Analysis of variance of the effects of diet and OCA on serum folates at week 2

BIOAVAILABILITY OF FA FROM OJUICE
T=2

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SERUM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	81.31083516	16.26216703	1.41	0.2333	0.115756	39.9942
ERROR	54	621.12516484	11.50231787		STD DEV		SERUM MEAN
CORRECTED TOTAL	59	702.43600000			3.39150673		8.48000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	31.69171303	1.38	0.2609	2	28.01057325	1.22	0.3039
OCA	1	49.40765611	4.30	0.0430	1	49.52171846	4.31	0.0428
DIET*OCA	2	0.21146603	0.01	0.9909	2	0.21146603	0.01	0.9909

BIOAVAILABILITY OF FA FROM OJUICE
T=2

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=11.5023

GROUPING	MEAN	N	DIET
A	9.361905	21	2
A			
A	8.405000	20	3
A			
A	7.594211	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=2

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=11.5023

GROUPING	MEAN	N	OCA
A	9.161538	39	1
B	7.214286	21	2

Appendix Table Bbv. Analysis of variance of the effects of diet and OCA on serum folates at week 5

BIOAVAILABILITY OF FA FROM OJUICE
T=5

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SERUM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	3548.13610154	709.62722031	2.00	0.0931	0.158642	100.1820
ERROR	53	18817.52966117	355.04772946		STD DEV		SERUM MEAN
CORRECTED TOTAL	58	22365.66576271			18.84271025		18.80847458

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	1230.92318375	1.73	0.1865	2	679.02000706	0.96	0.3909
OCA	1	1477.45132910	4.16	0.0464	1	1437.21841271	3.96	0.0517
DIET*OCA	2	839.76158868	1.18	0.3144	2	839.76158868	1.18	0.3144

BIOAVAILABILITY OF FA FROM OJUICE
T=5

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=53 MS=355.048

GROUPING	MEAN	N	DIET
A	25.065000	20	3
A			
A	16.665000	20	2
A			
A	14.479947	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=5

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=53 MS=355.048

GROUPING	MEAN	N	OCA
A	22.369231	39	1
B	11.865000	20	2

Appendix Table Bbvi. Analysis of variance of the effects of diet and OCA on serum folates at week 9

BIOAVAILABILITY OF FA FROM OJUICE
T=9

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SERUM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	2371.70358251	474.34071650	2.18	0.0700	0.176240	102.0354
ERROR	51	11085.49937363	217.36272301		STD DEV		SERUM MEAN
CORRECTED TOTAL	56	13457.20295614			14.74322634		14.44912281

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	1407.93298246	3.24	0.0474	2	863.68428208	1.99	0.1476
OCA	1	172.10938916	0.79	0.3777	1	177.74148515	0.82	0.3701
DIET*OCA	2	791.66121190	1.82	0.1722	2	791.66121190	1.82	0.1722

BIOAVAILABILITY OF FA FROM OJUICE
T=9

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=51 MS=217.363

GROUPING	MEAN	N	DIET
A	20.515789	19	3
A A			
B A	14.489474	19	2
B B			
B	8.342105	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=9

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=51 MS=217.363

GROUPING	MEAN	N	OCA
A	15.636842	38	1
A A			
A	11.673684	19	2

Appendix Table Bbvii. Analysis of variance of the effects of diet and OCA on serum folates at week 11

BIOAVAILABILITY OF FA FROM OJUICE
T=11

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: SERUM

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	3372.09942211	794.41988442	2.40	0.0492	0.190507	108.8062
ERROR	51	16967.08934982	330.72724215		STD DEV		SERUM MEAN
CORRECTED TOTAL	56	20339.18877193			18.18590779		16.71403509

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	1860.86982456	2.81	0.0693	2	1366.58238483	2.07	0.1372
OCA	1	21.06096711	0.06	0.8018	1	20.23122093	0.06	0.8056
DIET*OCA	2	2090.16863045	3.16	0.0508	2	2090.16863045	3.16	0.0508

BIOAVAILABILITY OF FA FROM OJUICE
T=11

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=51 MS=330.727

GROUPING	MEAN	N	DIET
A	23.542105	19	3
A			
B A	17.042105	19	2
B			
B	9.557895	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=11

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE SERUM

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=51 MS=330.727

GROUPING	MEAN	N	OCA
A	17.331579	38	1
A			
A	15.478947	19	2

Appendix Table Bbviii. Analysis of variance of the effects of diet and OCA on erythrocyte folates at week 0

BIOAVAILABILITY OF FA FROM OJUICE
T=0

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RBC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	49956.69578755	9991.33915751	0.63	0.6832	0.054753	28.0204
ERROR	54	862440.28754579	15971.11643603		STD DEV		RBC MEAN
CORRECTED TOTAL	59	912396.98333333			126.37688252		451.01666667

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	9515.59223058	0.30	0.7413	2	5942.57654375	0.19	0.8308
OCA	1	29824.85234930	1.87	0.1774	1	29912.66618910	1.87	0.1768
DIET*OCA	2	10516.25120767	0.33	0.7209	2	10516.25120767	0.33	0.7209

BIOAVAILABILITY OF FA FROM OJUICE
T=0

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=15971.1

GROUPING	MEAN	N	DIET
A	464.550000	20	3
A			
A	453.904762	21	2
A			
A	433.578947	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=0

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=15971.1

GROUPING	MEAN	N	OCA
A	481.047619	21	2
A			
A	434.346154	39	1

Appendix Table Bbix. Analysis of variance of the effects of diet and OCA on erythrocyte folates at week 2

BIOAVAILABILITY OF FA FROM OJUICE
T=2

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RBC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	23738.25073260	4747.65014652	0.55	0.7371	0.048757	30.9748
ERROR	54	463130.73260073	8576.49504816		STD DEV		RBC MEAN
CORRECTED TOTAL	59	486868.98333333			92.60936804		298.98333333

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	6999.15929825	0.41	0.6670	2	10856.86655023	0.63	0.5346
OCA	1	10173.22854884	1.19	0.2804	1	10117.61176226	1.18	0.2822
DIET*OCA	2	6540.85278552	0.38	0.6848	2	6540.85278552	0.38	0.6848

BIOAVAILABILITY OF FA FROM OJUICE
T=2

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=8576.5

GROUPING	MEAN	N	DIET
A	311.052632	19	1
A A			
A A	301.666667	21	2
A	284.700000	20	3

BIOAVAILABILITY OF FA FROM OJUICE
T=2

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=54 MS=8576.5

GROUPING	MEAN	N	OCA
A	308.461539	39	1
A A			
A	281.380952	21	2

Appendix Table Bbx. Analysis of variance of the effects of diet and OCA on erythrocyte folates at week 5

BIOAVAILABILITY OF FA FROM OJUICE
T=5

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RBC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	30263.37871037	6052.67574207	0.48	0.7935	0.043850	38.3889
ERROR	52	659898.96611722	12690.36473302		STD DEV		RBC MEAN
CORRECTED TOTAL	57.	690162.34482759			112.65151900		293.44827586

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	18779.22903911	0.74	0.4821	2	12703.74868297	0.50	0.6091
OCA	1	9778.76595736	0.77	0.3841	1	9957.72870545	0.79	0.3796
DIET*OCA	2	1705.38371490	0.07	0.9351	2	1705.38371490	0.07	0.9351

BIOAVAILABILITY OF FA FROM OJUICE
T=5

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=52 MS=12690.4

GROUPING	MEAN	N	DIET
A	316.210526	19	2
A			
A	292.400000	20	3
A			
A	271.789474	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=5

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=52 MS=12690.4

GROUPING	MEAN	N	OCA
A	303.447368	38	1
A			
A	274.450000	20	2

Appendix Table Bbxi. Analysis of variance of the effects of diet and OCA on erythrocyte folates at week 9

BIOAVAILABILITY OF FA FROM OJUICE
T=9

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RBC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	72230.02241180	14446.00448236	1.74	0.1422	0.145479	31.5313
ERROR	51	424269.03021978	8319.00059254		STD DEV		RBC MEAN
CORRECTED TOTAL	56	496499.05263158			91.20855648		289.26315789

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	62954.00000000	3.78	0.0294	2	58752.80991180	3.53	0.0366
OCA	1	5945.80021930	0.71	0.4018	1	5928.10140954	0.71	0.4025
DIET*OCA	2	3330.22219250	0.20	0.8192	2	3330.22219250	0.20	0.8192

BIOAVAILABILITY OF FA FROM OJUICE
T=9

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=51 MS=8319

GROUPING	MEAN	N	DIET
A	323.631579	19	2
A			
B A	299.842105	19	3
B			
B	244.315789	19	1

BIOAVAILABILITY OF FA FROM OJUICE
T=9

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=51 MS=8319

GROUPING	MEAN	N	OCA
A	297.657895	38	1
A			
A	272.473684	19	2

Appendix Table Bbxii. Analysis of variance of the effects of diet and OCA on erythrocyte folates at week 11

BIOAVAILABILITY OF FA FROM OJUICE
T=11

GENERAL LINEAR MODELS PROCEDURE

DEPENDENT VARIABLE: RBC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE	PR > F	R-SQUARE	C.V.
MODEL	5	83305.55219780	16661.11043956	1.33	0.2675	0.117266	37.9633
ERROR	50	624932.37637363	12496.64752747		STD DEV		RBC MEAN
CORRECTED TOTAL	55	707837.92857143			111.78840516		294.46428571

SOURCE	DF	TYPE I SS	F VALUE	PR > F	DF	TYPE IV SS	F VALUE	PR > F
DIET	2	70472.66541353	2.82	0.0691	2	51394.98976230	2.06	0.1386
OCA	1	7446.89390093	0.60	0.4438	1	7694.17207845	0.62	0.4364
DIET*OCA	2	5096.00288334	0.20	0.8165	2	5096.00288334	0.20	0.8165

BIOAVAILABILITY OF FA FROM OJUICE
T=11

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=50 MS=12496.6

GROUPING	MEAN	N	DIET
A	346.000000	18	3
B	270.789474	19	1
B			
B	269.315789	19	2

BIOAVAILABILITY OF FA FROM OJUICE
T=11

GENERAL LINEAR MODELS PROCEDURE

DUNCAN'S MULTIPLE RANGE TEST FOR VARIABLE RBC

MEANS WITH THE SAME LETTER ARE NOT SIGNIFICANTLY DIFFERENT.

ALPHA LEVEL=.05 DF=50 MS=12496.6

GROUPING	MEAN	N	OCA
A	302.918919	37	1
A			
A	278.000000	19	2

Appendix Table Bbxiii. Unpaired Student's t test of the effect of OCA on serum and erythrocyte folates at blood sampling weeks

T=0

TTEST PROCEDURE

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	16.50512821	16.83481588	2.69572799	6.30000000	80.00000000	UNEQUAL	2.7728	44.4	0.0081
2	21	5.70476190	3.68679755	0.80452518	4.00000000	18.00000000	EQUAL	2.0887	58.0	0.0411

FOR HO: VARIANCES ARE EQUAL, F'= 20.85 WITH 38 AND 20 DF PROB > F'= 0.0001

VARIABLE: FSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	1.69241432	1.16208927	0.18608321	0.80666667	6.72268908	UNEQUAL	2.3735	45.6	0.0219
2	21	1.22760665	0.27957746	0.06100880	0.84615385	1.94000000	EQUAL	1.7985	59.0	0.0773

FOR HO: VARIANCES ARE EQUAL, F'= 17.28 WITH 38 AND 20 DF PROB > F'= 0.0001

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	434.46615385	131.35759519	21.03404321	187.00000000	754.00000000	UNEQUAL	-1.4726	48.9	0.1473
2	21	481.04761905	106.67168143	23.27766927	220.00000000	616.00000000	EQUAL	-1.3832	58.0	0.1719

FOR HO: VARIANCES ARE EQUAL, F'= 1.52 WITH 38 AND 20 DF PROB > F'= 0.3221

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	1.54479940	0.65150117	0.10432369	0.53976311	2.98785425	UNEQUAL	-1.5400	46.8	0.1303
2	21	1.79177894	0.55810604	0.12180160	0.92039323	2.69696970	EQUAL	-1.4696	58.0	0.1471

FOR HO: VARIANCES ARE EQUAL, F'= 1.36 WITH 38 AND 20 DF PROB > F'= 0.4653

T=2

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	9.16153846	3.55564536	0.56934291	5.00000000	23.00000000	UNEQUAL	2.2797	48.5	0.0271
2	21	7.21428571	2.91702490	0.63674342	3.70000000	15.00000000	EQUAL	2.1479	58.0	0.0359

FOR HO: VARIANCES ARE EQUAL, F'= 1.48 WITH 38 AND 20 DF PROB > F'= 0.3476

continued

B30 contd.

Appendix Table Bbxiii (continued)

T=2

TTEST PROCEDURE

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	21	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	58.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	309.46153846	101.17608047	16.20128309	185.00000000	632.00000000	UNEQUAL	1.2469	55.6	0.2177
2	21	281.38095238	66.28036955	14.46349694	195.00000000	443.00000000	EQUAL	1.1034	58.0	0.2744

FOR HO: VARIANCES ARE EQUAL, F'= 2.33 WITH 38 AND 20 DF PROB > F'= 0.0464

VARIABLE: RRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	21	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	58.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

T=5

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	22.36923077	23.07261026	3.69457448	5.90000000	91.00000000	UNEQUAL	2.6738	47.0	0.0103
2	20	11.86500000	5.97285747	1.33557153	5.00000000	32.00000000	EQUAL	1.9942	57.0	0.0509

FOR HO: VARIANCES ARE EQUAL, F'= 14.92 WITH 38 AND 19 DF PROB > F'= 0.0001

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	39	2.46739621	2.50071098	0.40043423	0.62765957	12.28571429	UNEQUAL	1.9441	43.2	0.0584
2	20	1.66140103	0.48008300	0.10734982	0.78125000	2.66666667	EQUAL	1.4222	57.0	0.1604

FOR HO: VARIANCES ARE EQUAL, F'= 27.13 WITH 38 AND 19 DF PROB > F'= 0.0001

continued

B30 contd.

Appendix Table Bbxiii (continued)

STATISTICAL ANALYSIS SYSTEM 9

T=5

TTEST PROCEDURE

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	303.44736842	119.14145519	19.32723618	142.00000000	670.00000000	UNEQUAL	1.0392	48.9	0.3038
2	20	274.45000000	90.01197289	20.12728902	109.00000000	524.00000000	EQUAL	0.9532	56.0	0.3446

FOR HO: VARIANCES ARE EQUAL, F'= 1.75 WITH 37 AND 19 DF PROB > F'= 0.1932

VARIABLE: RBDC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	1.01870214	0.38303653	0.06213673	0.41040462	2.10810811	UNEQUAL	0.7078	54.3	0.4821
2	20	0.96078926	0.23809440	0.05323953	0.55612245	1.41422594	EQUAL	0.6151	56.0	0.5410

FOR HO: VARIANCES ARE EQUAL, F'= 2.59 WITH 37 AND 19 DF PROB > F'= 0.0299

T=9

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	15.83684211	18.26768556	2.96340991	4.80000000	100.00000000	UNEQUAL	1.2338	52.7	0.2228
2	19	11.67368421	7.03347884	1.61359071	4.70000000	30.00000000	EQUAL	0.9551	55.0	0.3437

FOR HO: VARIANCES ARE EQUAL, F'= 6.75 WITH 37 AND 18 DF PROB > F'= 0.0001

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	1.68635173	1.27304507	0.20651518	0.48260870	6.66666667	UNEQUAL	0.4070	54.2	0.6856
2	19	1.58736779	0.54993431	0.12614273	0.50537634	2.96296296	EQUAL	0.3214	55.0	0.7491

FOR HO: VARIANCES ARE EQUAL, F'= 5.36 WITH 37 AND 18 DF PROB > F'= 0.0004

VARIABLE: EDC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	297.65789474	97.21897165	15.77009974	145.00000000	532.00000000	UNEQUAL	0.9844	39.6	0.3309
2	19	272.47368421	87.79987878	20.14267362	185.00000000	550.00000000	EQUAL	0.9511	55.0	0.3457

FOR HO: VARIANCES ARE EQUAL, F'= 1.23 WITH 37 AND 18 DF PROB > F'= 0.6574

continued

B30 contd.

Appendix Table Bbxiii (continued)

T=9

TTEST PROCEDURE

VARIABLE: PPAC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	1.02908659	0.33305458	0.05402853	0.24534687	1.77358491	UNEQUAL	0.5195	34.1	0.6068
2	19	0.97831095	0.35502883	0.08144920	0.50948509	1.84615385	EQUAL	0.5309	55.0	0.5976

FOR HO: VARIANCES ARE EQUAL, F'= 1.14 WITH 18 AND 37 DF PROB > F'= 0.7183

T=11

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	17.33157895	19.08534326	3.09605151	8.13333333	95.00000000	UNEQUAL	0.3328	34.4	0.7413
2	19	15.47894737	20.16394495	4.62590329	5.00000000	95.00000000	EQUAL	0.3391	55.0	0.7358

FOR HO: VARIANCES ARE EQUAL, F'= 1.12 WITH 18 AND 37 DF PROB > F'= 0.7515

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	38	1.85842673	1.33624807	0.21676806	0.52173913	6.83453237	UNEQUAL	0.0545	40.2	0.9568
2	19	1.83946616	1.18678809	0.27226786	0.94339623	6.33333333	EQUAL	0.0523	55.0	0.9584

FOR HO: VARIANCES ARE EQUAL, F'= 1.27 WITH 37 AND 18 DF PROB > F'= 0.6011

VARIABLE: PDC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	37	332.91891892	123.05946504	20.23085156	157.00000000	612.00000000	UNEQUAL	0.8487	46.3	0.4004
2	19	278.00000000	92.75774900	21.28008705	151.00000000	517.00000000	EQUAL	0.7754	54.0	0.4415

FOR HO: VARIANCES ARE EQUAL, F'= 1.76 WITH 36 AND 18 DF PROB > F'= 0.2015

VARIABLE: RPAC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	37	1.02219584	0.37011744	0.06084693	0.32318105	2.05911330	UNEQUAL	0.0671	29.6	0.9470
2	19	1.01382673	0.47458206	0.10887659	0.46808511	2.65128205	EQUAL	0.0727	54.0	0.9423

FOR HO: VARIANCES ARE EQUAL, F'= 1.64 WITH 18 AND 36 DF PROB > F'= 0.2006

Appendix Table Bbxiv. Unpaired Student's t test of the effect of diet and OCA on serum and erythrocyte folates at blood sampling weeks

T=0 DIET=1

TTEST PROCEDURE

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	11.63333333	2.95768132	0.85380905	8.00000000	18.00000000	UNEQUAL	3.3739	15.9	0.0039
2	7	7.64285714	2.16553084	0.81849372	5.50000000	11.20000000	EQUAL	3.1022	17.0	0.0065

FOR HO: VARIANCES ARE EQUAL, F'= 1.87 WITH 11 AND 6 DF PROB > F'= 0.4585

VARIABLE: FSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.49737516	0.24704307	0.07131519	1.03278689	1.89473684	UNEQUAL	1.4054	11.4	0.1866
2	7	1.22829189	0.27939158	0.10560309	1.04545455	1.83606557	EQUAL	1.4543	17.0	0.1641

FOR HO: VARIANCES ARE EQUAL, F'= 1.28 WITH 6 AND 11 DF PROB > F'= 0.6835

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	407.83333333	145.21885260	41.92107102	198.00000000	738.00000000	UNEQUAL	-1.2181	16.1	0.2407
2	7	477.71428571	103.61099408	39.16127478	296.00000000	575.00000000	EQUAL	-1.1128	17.0	0.2813

FOR HO: VARIANCES ARE EQUAL, F'= 1.96 WITH 11 AND 6 DF PROB > F'= 0.4204

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.47191332	0.79255311	0.22879038	0.53976311	2.98785425	UNEQUAL	-0.4138	17.0	0.6842
2	7	1.58978636	0.44913827	0.16974697	0.95176849	2.17200000	EQUAL	-0.3586	17.0	0.7243

FOR HO: VARIANCES ARE EQUAL, F'= 3.11 WITH 11 AND 6 DF PROB > F'= 0.1751

T=0 DIET=2

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	16.06428571	11.94626476	3.19277356	6.30000000	49.00000000	UNEQUAL	1.4451	19.0	0.1647
2	7	10.54285714	5.55213387	2.09850935	4.00000000	18.00000000	EQUAL	1.1510	19.0	0.2640

FOR HO: VARIANCES ARE EQUAL, F'= 4.63 WITH 13 AND 6 DF PROB > F'= 0.0702

continued

B31 cont'd.

Appendix Table Bbxiv (continued)

T=0 DIET=2

TTEST PROCEDURE

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	1.54741338	0.63852374	0.17065265	0.92086331	3.15315315	UNEQUAL	0.9808	18.5	0.3394
2	7	1.32825905	0.36466885	0.13783197	0.88333333	1.94000000	EQUAL	0.8204	19.0	0.4222

FOR HO: VARIANCES ARE EQUAL, F'= 3.07 WITH 13 AND 6 DF PROB > F'= 0.1771

VARIABLE: RDC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	432.92857143	155.14532358	41.46433186	187.00000000	754.00000000	UNEQUAL	-1.3177	18.6	0.2036
2	7	495.85714286	62.69045146	23.69491444	408.00000000	602.00000000	EQUAL	-1.0215	19.0	0.3198

FOR HO: VARIANCES ARE EQUAL, F'= 6.12 WITH 13 AND 6 DF PROB > F'= 0.0354

VARIABLE: PRDC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	1.46749676	0.57301366	0.15314434	0.82768362	2.34716981	UNEQUAL	-1.4675	10.8	0.1707
2	7	1.89209332	0.64948678	0.24544293	0.92099323	2.69696970	EQUAL	-1.5333	19.0	0.1417

FOR HO: VARIANCES ARE EQUAL, F'= 1.28 WITH 6 AND 13 DF PROB > F'= 0.6592

T=0 DIET=3

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	21.47692308	26.15537401	7.25419555	6.40000000	80.00000000	UNEQUAL	1.8580	12.2	0.0874
2	7	7.92857143	1.95764679	0.73992094	4.80000000	10.50000000	EQUAL	1.3514	18.0	0.1933

FOR HO: VARIANCES ARE EQUAL, F'= 178.51 WITH 12 AND 6 DF PROB > F'= 0.0001

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	2.11598293	1.86474668	0.51718767	0.80666667	6.72268908	UNEQUAL	1.9007	12.3	0.0810
2	7	1.12626902	0.16031644	0.06059392	0.84615385	1.32051282	EQUAL	1.3840	18.0	0.1833

FOR HO: VARIANCES ARE EQUAL, F'= 135.30 WITH 12 AND 6 DF PROB > F'= 0.0001

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=0 DIFT=3

TTEST PROCEDURE

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	461.84615385	87.80361245	24.35234053	310.00000000	612.00000000	UNEQUAL	-0.1244	8.2	0.9040
2	7	469.57142857	151.13444031	57.12344909	220.00000000	616.00000000	EQUAL	-0.1459	18.0	0.8856

FOR HO: VARIANCES ARE EQUAL, F'= 2.96 WITH 6 AND 12 DF PROB > F'= 0.1034

VARIABLE: RRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.69532782	0.61303117	0.17001593	0.83333333	2.56372549	UNEQUAL	-0.7095	12.9	0.4906
2	7	1.89345713	0.58613982	0.22154003	1.12820513	2.61971831	EQUAL	-0.6995	18.0	0.4932

FOR HO: VARIANCES ARE EQUAL, F'= 1.09 WITH 12 AND 6 DF PROB > F'= 0.9659

T=2 DIFT=1

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	8.35000000	1.92900815	0.55711640	5.70000000	12.20000000	UNEQUAL	2.6143	15.4	0.0193
2	7	6.27142857	1.50079344	0.56724640	4.60000000	9.30000000	EQUAL	2.4413	17.0	0.0259

FOR HO: VARIANCES ARE EQUAL, F'= 1.65 WITH 11 AND 6 DF PROB > F'= 0.5556

VARIABLE: PSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	7	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	17.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

VARIABLE: EBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	313.66666667	107.22731448	30.95385944	194.00000000	591.00000000	UNEQUAL	0.2098	14.7	0.8367
2	7	306.57142857	36.02710620	13.61696621	250.00000000	369.00000000	EQUAL	0.1679	17.0	0.9687

FOR HO: VARIANCES ARE EQUAL, F'= 8.86 WITH 11 AND 6 DF PROB > F'= 0.0143

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=2 DIET=1

TTEST PROCEDURE

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	7	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	17.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

T=2 DIET=2

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	9.95714286	4.46830105	1.19420369	6.00000000	23.00000000	UNEQUAL	0.8743	12.3	0.3987
2	7	8.17142857	4.38357323	1.65683495	3.70000000	15.00000000	EQUAL	0.8685	19.0	0.3960

FOR HO: VARIANCES ARE EQUAL, F'= 1.04 WITH 13 AND 6 DF PROB > F'= 1.0000

VARIABLE: FSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	7	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	19.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	307.28571429	77.78612549	20.78921650	200.00000000	431.00000000	UNEQUAL	0.3905	9.8	0.7046
2	7	290.42857143	100.08805647	37.82972952	196.00000000	443.00000000	EQUAL	0.4261	19.0	0.6748

FOR HO: VARIANCES ARE EQUAL, F'= 1.66 WITH 6 AND 13 DF PROB > F'= 0.4191

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	7	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	19.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=2 DIET=3

TTEST PROCEDURE

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	9.05384615	3.68501818	1.02204015	5.00000000	15.00000000	UNEQUAL	1.4090	17.7	0.1762
2	7	7.20000000	2.19241115	0.82865353	4.10000000	10.70000000	EQUAL	1.2114	18.0	0.2414

FOR HO: VARIANCES ARE EQUAL, F'= 2.83 WITH 12 AND 6 DF PROB > F'= 0.2117

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	7	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	18.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	304.92307692	123.61125996	34.28359508	185.00000000	632.00000000	UNEQUAL	1.5808	14.9	0.1349
2	7	247.14285714	33.53321026	12.67436214	195.00000000	282.00000000	EQUAL	1.1993	18.0	0.2460

FOR HO: VARIANCES ARE EQUAL, F'= 13.59 WITH 12 AND 6 DF PROB > F'= 0.0044

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.00000000	0	0	1.00000000	1.00000000	UNEQUAL	.	.	.
2	7	1.00000000	0	0	1.00000000	1.00000000	EQUAL	.	18.0	.

NOTE: ALL VALUES ARE THE SAME FOR ONE CLASS LEVEL.

T=5 DIET=1

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	17.59166667	23.91007617	6.90224446	5.00000000	91.00000000	UNEQUAL	1.2135	11.4	0.2496
2	7	9.14285714	2.41236892	0.91178975	5.00000000	13.00000000	EQUAL	0.9211	17.0	0.3699

FOR HO: VARIANCES ARE EQUAL, F'= 98.24 WITH 11 AND 6 DF PROB > F'= 0.0001

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=5 DIET=1

TTEST PROCEDURE

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	2.03959679	2.53326392	0.73129030	0.62765957	9.89130435	UNEQUAL	0.7017	12.3	0.4960
2	7	1.51132618	0.47336067	0.17891352	0.78125000	2.13114754	EQUAL	0.5400	17.0	0.5962

FOR HO: VARIANCES ARE EQUAL, F'= 28.64 WITH 11 AND 6 DF PROB > F'= 0.0005

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	276.41666667	96.26521918	27.78937510	142.00000000	470.00000000	UNEQUAL	0.3520	16.9	0.7292
2	7	263.85714286	59.22394947	22.38454885	182.00000000	341.00000000	EQUAL	0.3105	17.0	0.7600

FOR HO: VARIANCES ARE EQUAL, F'= 2.64 WITH 11 AND 6 DF PROB > F'= 0.2442

VARIABLE: RRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	0.93121555	0.35337203	0.10200372	0.41040462	1.73431734	UNEQUAL	0.5890	16.4	0.5639
2	7	0.86128465	0.16074485	0.06075584	0.57053292	1.08681672	EQUAL	0.4904	17.0	0.6302

FOR HO: VARIANCES ARE EQUAL, F'= 4.83 WITH 11 AND 6 DF PROB > F'= 0.0655

T=5 DIET=2

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	17.14428571	15.88107762	4.24439653	8.10000000	63.00000000	UNEQUAL	0.2939	16.0	0.7726
2	6	15.50000000	9.17888882	3.74726567	8.00000000	32.00000000	EQUAL	0.2379	18.0	0.8146

FOR HO: VARIANCES ARE EQUAL, F'= 2.99 WITH 13 AND 5 DF PROB > F'= 0.2333

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	14	1.74971419	1.39213495	0.37206372	0.76258993	6.23188406	UNEQUAL	-0.3158	17.9	0.7558
2	6	1.89158951	0.61662267	0.25173515	0.90666667	2.66666667	EQUAL	-0.2370	18.0	0.8153

FOR HO: VARIANCES ARE EQUAL, F'= 5.10 WITH 13 AND 5 DF PROB > F'= 0.0831

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=5 DIET=2

TTEST PROCEDURE

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	329.00000000	142.16363811	39.42909498	182.00000000	670.00000000	UNEQUAL	0.5919	10.2	0.5668
2	6	288.50000000	136.96240360	55.91466713	109.00000000	524.00000000	EQUAL	0.5834	17.0	0.5673

FOR HO: VARIANCES ARE EQUAL, F'= 1.08 WITH 12 AND 5 DF PROB > F'= 1.0000

VARIABLE: PROC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.06941571	0.39949983	0.11080132	0.59033079	1.89265537	UNEQUAL	0.9665	14.9	0.3492
2	6	0.92323169	0.25220480	0.10296218	0.55612245	1.18284424	EQUAL	0.8172	17.0	0.4251

FOR HO: VARIANCES ARE EQUAL, F'= 2.51 WITH 12 AND 5 DF PROB > F'= 0.3187

T=5 DIET=3

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	32.39461538	26.93220760	7.46986121	8.00000000	86.00000000	UNEQUAL	2.7477	12.9	0.0167
2	7	11.47142857	3.86036021	1.45907031	7.00000000	18.00000000	EQUAL	2.0182	18.0	0.0587

FOR HO: VARIANCES ARE EQUAL, F'= 48.68 WITH 12 AND 6 DF PROB > F'= 0.0001

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	3.63514632	3.09185064	0.85752509	1.39062500	12.28571429	UNEQUAL	2.3326	12.5	0.0371
2	7	1.61417146	0.32720594	0.12367222	1.26666667	2.00000000	EQUAL	1.7029	18.0	0.1058

FOR HO: VARIANCES ARE EQUAL, F'= 89.29 WITH 12 AND 6 DF PROB > F'= 0.0001

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	302.84615385	116.71802602	32.37200558	168.00000000	505.00000000	UNEQUAL	0.6791	16.8	0.5063
2	7	273.00000000	78.65324744	29.72813322	131.00000000	345.00000000	EQUAL	0.6031	18.0	0.5540

FOR HO: VARIANCES ARE EQUAL, F'= 2.20 WITH 12 AND 6 DF PROB > F'= 0.3422

continued

B31 contd.

Appendix Table Bbxiv (continued)

S T A T I S T I C A L A N A L Y S I S S Y S T E M 24

T=5 DIET=3

TTEST PROCEDURE

VARIABLE: PRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.04874542	0.40852605	0.11330474	0.48743719	2.10810811	UNEQUAL	-0.2918	17.3	0.7739
2	7	1.09248607	0.25975638	0.09817868	0.67179487	1.41422594	EQUAL	-0.2551	18.0	0.8015

FOR HO: VARIANCES ARE EQUAL, F'= 2.47 WITH 12 AND 6 DF PROB > F'= 0.2753

T=9 DIET=1

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	8.80833333	1.99747947	0.57662265	4.80000000	12.40000000	UNEQUAL	1.4659	14.4	0.1641
2	7	7.54285714	1.69985994	0.64248667	4.70000000	9.40000000	EQUAL	1.4021	17.0	0.1789

FOR HO: VARIANCES ARE EQUAL, F'= 1.38 WITH 11 AND 6 DF PROB > F'= 0.7210

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.08435953	0.27447812	0.07923501	0.70491803	1.66176471	UNEQUAL	-1.1161	9.5	0.2919
2	7	1.27161373	0.39128081	0.14789025	0.50537634	1.77358491	EQUAL	-1.2281	17.0	0.2361

FOR HO: VARIANCES ARE EQUAL, F'= 2.03 WITH 6 AND 11 DF PROB > F'= 0.2919

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	252.58333333	71.46831254	20.63112474	145.00000000	388.00000000	UNEQUAL	0.7459	15.0	0.4673
2	7	230.14285714	57.93510327	21.89741077	185.00000000	325.00000000	EQUAL	0.7042	17.0	0.4908

FOR HO: VARIANCES ARE EQUAL, F'= 1.52 WITH 11 AND 6 DF PROB > F'= 0.6291

VARIABLE: PRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	0.88736216	0.35907740	0.10365672	0.24534687	1.57085020	UNEQUAL	1.0078	17.0	0.3277
2	7	0.75877251	0.19687510	0.07441179	0.50948509	1.01880878	EQUAL	0.8676	17.0	0.3977

FOR HO: VARIANCES ARE EQUAL, F'= 3.33 WITH 11 AND 6 DF PROB > F'= 0.1523

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=9 DIET=2

TTEST PROCEDURE

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	13.04615385	2.86199679	0.79377509	8.50000000	20.00000000	UNEQUAL	-1.1132	5.4	0.3131
2	6	17.61666667	9.86720156	4.02826817	6.50000000	30.00000000	EQUAL	-1.5785	17.0	0.1329

FOR HO: VARIANCES ARE EQUAL, F'= 11.89 WITH 5 AND 12 DF PROB > F'= 0.0005

VARIABLE: FSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.46387249	0.45314864	0.12568082	0.48260870	2.02702703	UNEQUAL	-2.1512	7.5	0.0660
2	6	2.07837836	0.62833635	0.25651724	1.25000000	2.96296296	EQUAL	-2.4368	17.0	0.0261

FOR HO: VARIANCES ARE EQUAL, F'= 1.92 WITH 5 AND 12 DF PROB > F'= 0.3240

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	324.00000000	100.10494493	27.76411630	174.00000000	505.00000000	UNEQUAL	0.0205	8.3	0.9841
2	6	322.83333333	121.63127339	49.65575943	200.00000000	550.00000000	EQUAL	0.0221	17.0	0.9826

FOR HO: VARIANCES ARE EQUAL, F'= 1.48 WITH 5 AND 12 DF PROB > F'= 0.5363

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.12393459	0.35923929	0.09963505	0.60451077	1.77358491	UNEQUAL	0.1569	9.7	0.8786
2	6	1.09591819	0.36301113	0.14819867	0.69977427	1.67173252	EQUAL	0.1575	17.0	0.8767

FOR HO: VARIANCES ARE EQUAL, F'= 1.02 WITH 5 AND 12 DF PROB > F'= 0.8951

T=9 DIET=3

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	25.11538462	29.42759040	8.16174539	8.00000000	100.00000000	UNEQUAL	1.7638	12.6	0.1021
2	6	10.55000000	3.07014879	1.25364898	6.70000000	16.00000000	EQUAL	1.1909	17.0	0.2500

FOR HO: VARIANCES ARE EQUAL, F'= 91.40 WITH 12 AND 5 DF PROB > F'= 0.0001

continued

B31 contd.

Appendix Table Bbxiv (continued)

S T A T I S T I C A L A N A L Y S I S S Y S T E M 26

T=9 DIET=3

TTEST PROCEDURE

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	2.46451607	1.91064476	0.52991751	0.59333333	6.66666667	UNEQUAL	1.8492	12.9	0.0875
2	6	1.40632028	0.25175388	0.10277909	1.01098901	1.68181818	EQUAL	1.2554	17.0	0.2263

FOR HO: VARIANCES ARE EQUAL, F'= 57.60 WITH 12 AND 5 DF PROB > F'= 0.0003

VARIABLE: FBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	312.92307692	106.78507094	29.61709952	173.00000000	532.00000000	UNEQUAL	1.0916	16.3	0.2909
2	6	271.50000000	58.10937962	23.72305489	207.00000000	360.00000000	EQUAL	0.8826	17.0	0.3898

FOR HO: VARIANCES ARE EQUAL, F'= 3.38 WITH 12 AND 5 DF PROB > F'= 0.1882

VARIABLE: PBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.06506114	0.25207433	0.06991294	0.64247312	1.49545455	UNEQUAL	-0.2852	6.8	0.7840
2	6	1.11683190	0.41033576	0.16751887	0.73404255	1.84615385	EQUAL	-0.3414	17.0	0.7369

FOR HO: VARIANCES ARE EQUAL, F'= 2.65 WITH 5 AND 12 DF PROB > F'= 0.1547

T=11 DIET=1

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	10.39166667	1.46129353	0.42183911	8.10000000	12.80000000	UNEQUAL	2.2369	8.6	0.0536
2	7	8.12857143	2.43290929	0.91955328	5.00000000	10.40000000	EQUAL	2.5542	17.0	0.0205

FOR HO: VARIANCES ARE EQUAL, F'= 2.77 WITH 6 AND 11 DF PROB > F'= 0.1361

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.29736128	0.31761703	0.09169814	0.88043478	1.88235294	UNEQUAL	0.0163	14.0	0.9872
2	7	1.29507556	0.28105330	0.10622816	0.94339623	1.62500000	EQUAL	0.0157	17.0	0.9876

FOR HO: VARIANCES ARE EQUAL, F'= 1.28 WITH 11 AND 6 DF PROB > F'= 0.7988

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=11 DIET=1

TTEST PROCEDURE

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	277.16666667	100.52121742	29.01797597	157.00000000	431.00000000	UNEQUAL	0.4438	16.4	0.6630
2	7	259.85714286	68.95512689	26.06258819	177.00000000	386.00000000	EQUAL	0.4015	17.0	0.6930

FOR HO: VARIANCES ARE EQUAL, F'= 2.13 WITH 11 AND 6 DF PROB > F'= 0.3668

VARIABLE: RRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	0.97134333	0.47479166	0.13706055	0.32318105	2.05911330	UNEQUAL	0.6917	16.8	0.4985
2	7	0.85754075	0.24076083	0.09099304	0.56913183	1.24115756	EQUAL	0.5867	17.0	0.5651

FOR HO: VARIANCES ARE EQUAL, F'= 3.89 WITH 11 AND 6 DF PROB > F'= 0.1082

T=11 DIET=2

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	12.25384615	2.28249709	0.63332814	8.80000000	17.00000000	UNEQUAL	-1.0749	5.0	0.3314
2	6	27.41666667	34.51680267	14.09142569	6.70000000	95.00000000	EQUAL	-1.6326	17.0	0.1209

FOR HO: VARIANCES ARE EQUAL, F'= 229.49 WITH 5 AND 12 DF PROB > F'= 0.0001

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	1.37729959	0.43032031	0.11934930	0.52173913	2.00000000	UNEQUAL	-1.4994	5.2	0.1918
2	6	2.57219328	1.92997875	0.78791053	1.10091743	6.33333333	EQUAL	-2.1863	17.0	0.0431

FOR HO: VARIANCES ARE EQUAL, F'= 20.12 WITH 5 AND 12 DF PROB > F'= 0.0001

VARIABLE: PBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	270.69230769	105.63962689	29.29916088	167.00000000	514.00000000	UNEQUAL	0.0906	11.0	0.9294
2	6	266.33333333	93.47655676	38.16164453	154.00000000	425.00000000	EQUAL	0.0864	17.0	0.9322

FOR HO: VARIANCES ARE EQUAL, F'= 1.28 WITH 12 AND 5 DF PROB > F'= 0.8380

continued

B31 contd.

Appendix Table Bbxiv (continued)

T=11 DIET=2

TTEST PROCEDURE

VARIABLE: RRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	0.93018413	0.23675709	0.06566469	0.50150150	1.30788804	UNEQUAL	-0.0212	8.0	0.9836
2	6	0.91211929	0.29854548	0.12188368	0.46408511	1.33838384	EQUAL	-0.0232	17.0	0.9818

FOR HO: VARIANCES ARE EQUAL, F'= 1.59 WITH 5 AND 12 DF PROB > F'= 0.4723

T=11 DIET=3

VARIABLE: SERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	28.91538462	29.94948097	8.30649149	9.70000000	95.00000000	UNEQUAL	1.9984	12.3	0.0683
2	6	12.11666667	2.22747989	0.90936446	9.20000000	16.00000000	EQUAL	1.3431	17.0	0.1969

FOR HO: VARIANCES ARE EQUAL, F'= 180.78 WITH 12 AND 5 DF PROB > F'= 0.0001

VARIABLE: RSERUM

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	13	2.85746043	1.89235710	0.52484543	1.26050420	6.83453237	UNEQUAL	2.0184	14.4	0.0627
2	6	1.74186140	0.42457737	0.17333238	1.10280374	2.24390244	EQUAL	1.4070	17.0	0.1774

FOR HO: VARIANCES ARE EQUAL, F'= 19.87 WITH 12 AND 5 DF PROB > F'= 0.0040

VARIABLE: RBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	363.58333333	146.00215337	42.14719127	185.00000000	612.00000000	UNEQUAL	0.8128	12.0	0.4321
2	6	310.83333333	120.89410428	49.35072832	151.00000000	517.00000000	EQUAL	0.7610	16.0	0.4578

FOR HO: VARIANCES ARE EQUAL, F'= 1.46 WITH 11 AND 5 DF PROB > F'= 0.7114

VARIABLE: FRBC

OCA	N	MEAN	STD DEV	STD ERROR	MINIMUM	MAXIMUM	VARIANCES	T	DF	PROB > |T|
1	12	1.19547771	0.33327593	0.09620847	0.65371025	1.81621622	UNEQUAL	-0.3339	6.1	0.7496
2	6	1.29786781	0.71323840	0.29117836	0.53928571	2.65128205	EQUAL	-0.4221	16.0	0.6785

FOR HO: VARIANCES ARE EQUAL, F'= 4.58 WITH 5 AND 11 DF PROB > F'= 0.0334