FOLATE STATUS AND KNOWLEDGE OF SIKH WOMEN OF CHILDBEARING AGE LIVING IN THE LOWER MAINLAND OF BRITISH COLUMBIA.

by:

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Sikh population is at a higher risk for having neural tube defects (NTD) affected pregnancies compared to the general Canadian population. The women capable of becoming pregnant are recommended to increase the consumption of folic acid to reduce the risk of NTD. This descriptive study of 45 Sikh women between the ages of 18 – 45 years living in the Lower Mainland, BC, assessed their folate status, knowledge level of folate and neural tube defects, and beliefs regarding diet and pregnancy. The folate intake was estimated using seven 24 hour dietary recalls conducted over a period of 4 weeks and included representative number of weekdays and weekend days. The red blood cell folate and plasma homocysteine analysis on the blood samples of these women were performed by the BC Biomedical Laboratories. An interviewer administered questionnaire was used to determine the level of folate knowledge and their beliefs regarding diet and pregnancy. Folic acid fortification increased folate intake by 121 ± 53 µg synthetic folic acid (SFA)/day from 291 ± 75 dietary folate equivalents (DFE)/day to 492 ± 132 DFE/day. For regular user, supplements contributed an average of 351 ± 267 µg SFA/day. The mean daily folate intake from all sources was 727 ± 420 DFE/day and 96% women were meeting the Estimated Average Requirement (EAR; 320 DFE/day). However, only 24% women were meeting the special recommendation of 400 µg SFA/day for women capable of becoming pregnant. The mean red blood cell folate level 958 ± 213 nmol/L and 56% women had levels > 906 nmol/L, which is
associated with reduced risk of NTD. The mean plasma homocysteine level was 7.3 ± 2.5 μmol/L. Only half of the women had heard of folate and the most common sources of this information were doctors, magazines/newspaper and school. Lack of awareness and belief that diet supplies enough folic acid were the most common reasons provided by the Sikh women for not taking a supplement. Most women believed that diet played an important role in pregnancy. Strategies are required to increase the knowledge of Sikh women regarding folate and neural tube defects.
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CHAPTER 1: INTRODUCTION

Neural tube defects (NTD) represent congenital defects of the central nervous system that arise from improper development during embryogenesis. Spina bifida and anencephaly are the most common NTDs (Forman et al., 1996; Hall et al., 1988). Approximately 1 in every 1000 births is affected with NTD (Provincial Health Officer’s Report, 1997). Even though British Columbia (BC) has one of the lowest rates of NTD in Canada (0.71 per 1000 live births), there is a high occurrence rate of NTD among the BC Sikh population. This population has demonstrated an occurrence rate of 3.87:1000 live births (Chambers et al., 1994; Hall et al., 1988; Baird, 1983), which is similar to the highest provincial rates found in Canada, namely Newfoundland and Quebec.

Increasing folic acid intake prior to pregnancy has been shown to reduce the incidence of NTD. A number of studies have reported a reduction in incidence of NTD affected pregnancy following the use of periconceptional folic acid supplements (Van Allen et al., 1993; MRC Vitamin Study Research Group, 1991; Smithells et al., 1983; Laurence et al., 1981; Smithells et al., 1981). Neural tube defects are a result of defective neural tube closure, which occurs between day 22 and 28 of gestation. Since most women are not aware that they are pregnant by this time, Canada and the United States recommend that all women capable of becoming pregnant should consume 400 μg/day of synthetic folic acid from supplements and/or fortified foods in addition to a varied diet to decrease NTD risk (Institute of Medicine, 1998).

There is no information available on the folate intakes and biochemical parameters related to folate status of Sikh women living in BC. Thus, we have no
indication that Sikh women of childbearing age, living in BC are consuming adequate amount of folate from food and/or supplements and meeting the special recommendation for reducing the risk of NTD. Low maternal red blood cell folate and high plasma homocysteine levels, parameters used to determine folate status, are also associated with the risk of NTD affected pregnancies. There is also a lack of information on the level of awareness of the recommendation to increase folic acid intake within this group and their attitudes and perceptions regarding diet during pregnancy and the use of supplements.

The primary aim of this project was to assess the folate status in Sikh women of childbearing age residing in the Lower Mainland, BC. In doing so, we assessed folate intakes, red blood cell folate levels and plasma homocysteine levels of these women. Secondly, we wanted to assess the awareness of the relationship between folate and NTD in this population, and their beliefs regarding diet during pregnancy and use of supplements.

The study results have provided documentation to assist community educators and health professionals in developing culturally relevant resources for increasing the awareness of folate for decreasing NTD risk among Sikh women of childbearing age.
CHAPTER 2: LITERATURE REVIEW

In the course of this chapter, the existing literature on NTD, the prevalence of NTD within the Sikh population, its etiology, folate biochemistry, folate intake and women's knowledge of the importance of adequate folate intake is reviewed. In addition the dietary practices and intakes of Sikh women will be discussed.

2.1 Background on Neural Tube Defects

Neural tube defects in humans are among the most common congenital malformations (Locksmith and Duff, 1998). The frequency of occurrence of NTD ranges from 1 per 1000 live births in low risk populations, up to 8 or more per 1000 in high-risk populations (Crockroft, 1991). Health Canada estimates that there are at least 400 NTD babies born each year in Canada, that is at the rate of approximately 1 per 1000 live births (Health Canada, 1995). Half of these infants are so seriously affected that they do not survive the neonatal period (Turner and McCourt, 1998). This estimation does not take into account the number of aborted or miscarried NTD-affected fetuses. Van Allen et al., (1997) have estimated that the fetal NTD incidence in Metropolitan Toronto could be as high as 1.78 per 1000, compared to the rate of 1 per 1000 live births, demonstrating that NTD affects more pregnancies than has been previously suggested (Van Allen et al., 1997).

Neural tube defects are a result of the failure of the process of neurulation. Neurulation (Figure 1) is a complex developmental process in all vertebrate embryos by which the flat neural plate of the early embryo is transformed into a tube that becomes the
brain cranially and spinal cord caudally (Locksmith and Duff, 1998; Crockroft, 1991). This process of neural tube closure in humans begins approximately on day 18 of fetal development and is over by day 28 of pregnancy (Northrup and Volick, 2000). There are two major theories surrounding the process of neural tube closure, and the timing. The traditional “zipper model” states that the neural folds first meet and fuse in the cervical region and then the tube closes in a continuous, bi-directional process. Van Allen and colleagues introduced the “multi-site closure model” in 1993, which states that the human neural fold fusion is initiated at five different sites and that the clinical types of neural tube defects differ depending on the site at which the closure fails (Van Allen et, 1993).

As the neural plate folds into a tube and subsequently develops into a spinal cord, bone and muscle form a protective covering around it. But, if a part of the neural plate fails to fuse into a tube due to abnormal neurulation, bone and muscle are unable to grow over this open section of the developing spinal column, and this results into a hole in the back through which the spinal cord and/or the meninges (nerve tissue) protrude out resulting into spina bifida (Figure 2). Anencephaly on the other hand is a result of abnormal development of brain and skull due to failure in closure of brain neural folds followed by degeneration of the brain tissue (Cockroft, 1991). Anencephaly and spina bifida are the two most common NTDs.

Neural tube defects can result in stillbirths, neonatal death, and severe disabilities. One half of the infants born with NTDs do not survive the neonatal period (Turner and McCourt, 1998). All infants with anencephaly are stillborn or die shortly thereafter. However, in North America, most infants with spina bifida now survive as a consequence
of extensive medical and surgical care available (Botto et al., 1999), but not without severe, life-long disabilities in most cases.

Figure 1: The neural plate folds to form a groove (a) and (b) into neural fold (c), which eventually forms the closed neural tube (d). {Source: www.spinabifida.org/Spina%20Bifida.htm accessed on 14 September 2003}
The cause of NTD in humans is considered to be multifactorial. Epidemiological studies reveal striking differences in the prevalence according to geographical area, season of birth, ethnic origin, and social class of the parents (Crockfort, 1991; Hall et al., 1988; Field and Kerr, 1981). Neural tube defects are hypothesized to occur in individuals with polygenic susceptibility to a particular trait, which becomes evident after exposure to certain environmental factors (Locksmith and Duff, 1998). Environmental factors include teratogen exposure and/or nutritional deficiency/ies (Locksmith and Duff, 1998). Maternal vitamin status, specifically folic acid deficiency, has been implicated in the development of NTD (Locksmith and Duff, 1998).

2.2 Prevalence of Neural Tube Defects in the Sikh Population

The incidence of NTD affected births is higher within the Sikh population of BC than in the non-Sikh population. During 1958-1984 the occurrence rate of NTD affected births among Sikh families in BC was 1 in 350 live births as compared to 1 in 795 live
births among non-Sikh families (Chambers et al., 1994). Following this, from 1985-1993, there was a decrease in the rate of NTD in non-Sikh births to 1 in 1415 live births as compared to a simultaneous increase in the rate of NTDs among Sikh births to 1 in 262 live births (Chambers et al., 1994).

A similarly high occurrence rate of NTD has been noted in Sikh populations elsewhere in the world. For instance, in India a higher rate of NTD occurrence is observed among the Sikhs when compared to the general population. In 1966 Stevenson et al., suggested that the Sikhs have an increased risk of NTD occurrence, compared to Hindus living in the same state of Punjab (Stevenson et al., 1966). Verma (1978) demonstrated a high rate of NTD in North India, and an even higher rate of occurrence in the cities of Chandigarh (8.8:1000 births), and Amritsar (8.0:1000 births) in Punjab, where there is a high proportion of Sikh residents (Verma, 1978). Verma suggested that the high occurrence rates are a general phenomenon of North India as a whole rather than just Punjab, or Sikhs in particular (Figure 3). However, it is also important to take into consideration that the author reported that the prevalence of anencephaly among Sikhs is higher than that of the Hindus when data from the same hospital were compared. This is very intriguing because there is historical and cultural evidence that both Sikhs and Hindus of Punjab have a similar genetic background (Verma, 1978). Hall et al., (1988) suggested that an increased incidence of NTDs among the Sikhs, even when compared to their Hindu neighbors from the same region in Punjab, could be due to the occurrence of a Mendelian disorder in this population (Hall et al., 1988). It is possible that this disorder arose either by migration into the population or by mutation (Hall et al., 1988). This disorder possibly did not spread to the Hindus having similar cultural practices, genetic
background, and living in the same region because of the prohibitions on intermarriage (Hall et al., 1988). Arranged marriages are still very common in the Sikh population, so the inbreeding may persist, perhaps explaining the high incidence of NTD in this population (Hall, 1994).

In 1959, Searle reported an occurrence rate of 6.5:1000 births for anencephalus among the offspring of Sikh mothers, compared to an overall rate of 0.77:1000 births in his study population from one hospital in Singapore (Searle, 1959). In the population from the same hospital, Stevenson et al., (1966) were unable to confirm the results of Searle. The authors attributed this lack of confirmation with the results of Searle (1959) to the fact that the proportion of all births to Indian mothers had fallen considerably from 16.5% in 1953-59, when Searle conducted his study, to 7.8% in 1963 when Stevenson et al., collected their data.

The immigrants of Indian and Pakistani origin in the United Kingdom (UK) demonstrate a higher rate of NTD (Leek and Lancashire, 1995; Michie et al., 1998) than their European counterparts. In Birmingham, UK, Leck (1969) showed an occurrence rate of 5.4:1000 total births within his population group of Indian or Pakistani origin, the majority being Punjabi Sikhs.

The type of lesion found in cases of spina bifida among Sikh immigrants in BC is anatomically and etiologically distinct (Hall et al., 1988). Hall et al., (1988) observed that high lesions, that is involving T11 or above (Figure 2), occurred in 31% of Sikh probands but only 14.7% of non-Sikh probands (Hall et al., 1988). The authors also observed that clinically, Sikhs with high lesions did better than others with similar lesions. That is, Sikh probands with lesions above T12 were all ambulatory. Furthermore, hydrocephalus
without spina bifida occurred in 6% of the siblings of Sikh NTD probands but only in 0.15% of siblings on non-Sikh probands. As a result, Hall (1994) suggested that a specific recessive gene is responsible for the high rate of NTD in the Sikh population (Hall, 1994).

Apart from genetic factors, there is a possibility of cultural and environmental factors playing a role in the high incidence of NTD among the Sikhs. For example, the Sikhs do not believe in abortions (Keena et al., 1992; Drakulic and Tanaka, 1981), and they also tend not to seek genetic counseling (Keena et al., 1992). Thus, it is possible that they do not terminate NTD-affected pregnancies (Van Allen, 1994), which can explain why the rate of NTD for the non-Sikh population has declined and that for the Sikh population has increased over the years. Diet is another important environmental factor that has been implicated in the etiology of NTD. It has been established that inadequacy of folic acid in a woman’s diet around the period of conception increases the risk of NTD-affected births. However, we do not know the dietary folate status of Sikh women in the Lower Mainland, BC.
Figure 3: Map of India depicting all the states and union territories. {Source: www.atrip2india.com/india_map.htm accessed on 14 September 2003}
2.3 Background on Folate

Wills and Mehta (1930, 1931) first treated pernicious anemia in Bombay, India by yeast or a yeast extract. The factor in yeast is what is now known as folic acid. In 1941, folic acid was isolated from spinach and acquired its name from the Latin word for leaf, folium (Hoffbrand, 2001). Folic acid is a water-soluble B-vitamin. It is composed of pteridine ring, paraaminobenzoic acid and glutamic acid (Figure 4). Naturally occurring folates are less bioavailable and incompletely utilized (Bailey, 1992) because, they are predominantly polyglutamates, and folate is absorbed in its monoglutamate form. Thus, the polyglutamates from folate need to be deconjugated before they can be absorbed as monoglutamate (Cuskelly et al., 1996; Bailey, 1992). Folate and folic acid are the preferred synonyms for this vitamin. Folate is a generic term used for many different naturally occurring compounds all of which exhibit vitamin like activity similar to that of pteroyl-L-glutamic acid. Naturally occurring folates are present in the polyglutamate form and are reduced to di-or tetra-hydro forms and they possess additional single carbon units such as methyl (-CH$_3$), formyl (-CHO), and methylene (=CH$_2$), attached to the N$_5$ and/or N$_{10}$ nitrogen atoms (Figure 4). Folic acid, on the other hand is used to denote the fully oxidized chemical compound (monoglutamyl pteroylglutamic acid), which is not present in natural foods. Sauberlich et al., (1987) indicated that food folate is approximately 50% bioavailable, whereas synthetic folic acid, which is present in monoglutamyl form, is 100% available when consumed as a supplement without food (Gregory, 1997).
Folate is also heat sensitive and a highly unstable vitamin (Locksmith and Duff P, 1998; Gregory, 1997; Steegers-Theunissen, 1995). Prolonged cooking, processing or storage can deteriorate the folate content of the food, thus even if a woman is able to identify a good source of folate, it is likely that she may not be getting enough (Scott, 1998). Besides this, dietary folate is normally accompanied with dietary fiber as part of mixed meals, and fiber may inhibit the absorption of folate from food (Gregory, 1997).

Folate provides single carbon units for the de novo synthesis of three out of four nucleotide bases of DNA, i.e. guanine, adenine and thymine (Locksmith and Duff, 1998; Steegers-Theunissen, 1995). The requirement of folate thus increases during periods of growth such as pregnancy. During pregnancy, factors such as poor diet, physiological hemodilution associated with pregnancy, increased plasma clearance rates and hormonal influences can have a negative impact on folate status (Locksmith and Duff, 1998).
2.4 Background on Folate and Neural Tube Defects

In 1952, Theirsch suggested an association between maternal lack of folate and malformations in their offsprings in a human study. In 1965, Hibbard and Smithells reported a possible link between folate deficiency and the development of human fetal malformations of the central nervous system, including NTD.

Daly et al., (1995) found that the risk of having NTD-affected offsprings was decreased by increasing the maternal red blood cell folate concentration from low (<149 ug/L) to normal (150-199 ug/L). Increasing the maternal red blood cell folate concentration to values greater than 300-400 ug/L further decreased the risk (Daly et al., 1995). Brown et al., (1997) demonstrated that red blood cell folate levels are sensitive to an individual's folate intake. Thus, there appears to be a link between folate intakes, folate status during pregnancy, and the susceptibility and prevention of NTD. However, the mechanisms underlying this relationship are not well understood. The absence of a good animal model is in part responsible for not being able to understand the relationship (Scott, 1998).

It has been suggested that maternal disturbance in homocysteine metabolism (Figure 5) may be responsible for NTD (Steegers-Theunissen et al., 1991). Recent studies have shown that hyperhomocysteinemia is linked with folate metabolism and NTD. A case control study by Mills et al., (1996), demonstrated that mothers of infants with NTD had significantly higher homocysteine levels than mothers without NTD affected infants. This was true even when controlled for vitamin B₁₂ status (Mills et al., 1996). Inadequate homocysteine metabolism may occur due to a defect in one of the following three enzymes: 1) cystathionine synthase, 2) methionine synthase, or 3) 5, 10 methylene
tetrahydrofolate reductase (Mills et al., 1996). A defect in methylene tetrahydrofolate reductase seems to be the most plausible one in humans.

![Folate-Homocysteine Metabolism Pathway](source.png)

Figure 5: Folate-Homocysteine Metabolism Pathway {Source: www.ds-health.com/abst/a0108.htm accessed on 14 September 2003}

This enzyme converts 5, 10-methylene tetrahydrofolate to 5-methyl tetrahydrofolate, the principle circulating form of folic acid (Locksmith and Duff, 1998). A specific mutation of the methylene tetrahydrofolate reductase gene, 677C → T (substitution of C - cytosine to T - thymine at nucleotide 677), causes the substitution of valine for alanine in the functional enzyme. It is an autosomal recessive mutation, which produces a thermolabile variant of 5, 10-methylene tetrahydrofolate reductase with reduced enzyme activity. The variant causes hyperhomocysteinemia and low red blood cell folate levels (Kang et al., 1991; Molly et al., 1997). Deficiency of folate as 5-methyl tetrahydrofolate results in the blocking of the remethylation of homocysteine. This block leads to elevated homocysteine levels, which may be embryotoxic (Steegers-Theunissen, 1995). The
frequency of the homozygous polymorphism 677C → T (i.e., T/T) is increased in mothers, fathers and patients with NTD (van der Put et al., 1995).

A deficiency of folate also leads to decreased methionine concentrations. Because methionine is an important donor of methyl groups, which are essential in the methylation of DNA and RNA, decreased methionine levels in addition to elevated homocysteine concentrations may disturb the development of the neural tube as one of its effects (Steegers-Theunissen, 1995). The polymorphism in association with the disturbed folate-homocysteine one carbon transfer has also been implicated in adult cardiovascular disease and colon cancer development risk (Andreassi et al., 2003).

Many studies have demonstrated the beneficial effect of folic acid supplements in reducing the risk of recurrence of NTD among mothers who have already given birth to a NTD-affected child (Laurence et al., 1981; Smithells et al., 1983; Smithells et al., 1981; MRC Vitamin Study Research Group, 1991). In 1981, Laurence et al., reported a reduction in the recurrence of NTD with daily supplementation of 4.0 mg of folic acid. Smithells et al., (1981, 1983) reported an 86% decline in the recurrence of NTD upon taking a daily supplement containing 0.36 mg of folic acid. The one study that is considered to be a landmark study demonstrating results similar to those mentioned above, was the UK Medical Research Council’s multicentre, randomized, double blind prevention trial (MRC Vitamin Study Research Group, 1991). The authors assigned the participating women randomly to receive a multivitamin supplement, folic acid supplement, both supplements, or none of the supplements. The supplements were administered starting from at least four weeks prior to conception, and were continued until 12 weeks gestation. The study concluded that a folic acid supplement at the level of
4 mg/day showed a 72% protective effect on the recurrence of NTD. Shaw et al., (1995) in a case-control study confirmed these results (Shaw et al., 1995).

Although the recurrence risk for NTD is around ten times the de novo occurrence risk, approximately 95% of women who deliver infants with NTD have no previous history of an affected pregnancy (Locksmith and Duff, 1991). The Hungarian randomized trial by Czeizel and Dudas in 1992 provided strong evidence in favor of the ability of periconceptional supplementation to prevent the occurrence of NTD. Women without a previously affected pregnancy were randomly assigned to receive a daily multivitamin supplement containing 0.8 mg of folic acid or a placebo containing trace elements. The supplements were ingested from at least one month prior to conception until the third month of gestation. The folic acid supplemented group demonstrated a significantly lower incidence of NTD (none of 2104 offspring) than the placebo group (6 of 2052 offspring) (Czeizel and Dudas, 1992). Other studies have reported similar results (Werler et al., 1993; Mulinare et al., 1988; Czeizel, 1998; Milunsky et al., 1989; Rasmussen et al., 1998). The study by Werler et al., (1993) conducted in Boston showed the first evidence of a statistically significant reduction in the occurrent NTD risk by using supplements containing 0.4 mg of folic acid, the amount of folic acid that is in most over the counter multivitamin preparations for adults.

These findings have led authorities all over the world to revise their recommendations for folate intake for women of childbearing age.
2.4.1 Recommendations for Women Capable of Becoming Pregnant to Reduce NTD Risk

As a consequence of the unequivocal findings from the studies discussed in the previous section, in 1993, Health and Welfare Canada suggested that all women of childbearing potential should consume foods high in folate and in addition, recommended a supplement of 400 μg folate per day (Health and Welfare Canada, 1993). It was soon discovered that the folic acid supplements were more effective in raising the blood folate status compared to the dietary folate (Cuskelly et al., 1996). Cuskelly et al., (1996) reported that although aggressive intervention with dietary folate or dietary advice significantly increased the intake of folate from food, this did not affect the folate status. Daly et al., in 1995 suggested that 400 ng/mL of red blood cell folate levels were associated with the prevention of folate-responsive NTD. Supplementing the diets of women of childbearing age with 400 μg of folic acid per day was reported to effectively raise the red blood cell folate to these levels (Brown et al., 1997). Thus, the recommendation for reducing NTD risk was revised as Canada and the United States established the new Dietary Reference Intakes (DRIs) for folate (Institute of Medicine, 1998). This new recommendation for all women capable of becoming pregnant is to consume 400 μg/day of synthetic folic acid from supplements and/or fortified foods in addition to folate from a varied diet to reduce the risk of NTD-affected pregnancy (Institute of Medicine, 1998).

The UK has similar recommendations (Centers for Disease Control and Prevention, 1992; Department of Health 1992). Australia and the Netherlands recommend an intake of 500 μg folic acid per day by women planning pregnancy.
(National Health and Medical Research Council, 1995; Health Council/Food and Nutrition Council Report Committee, 1993). Women who have had a previous pregnancy affected with NTD are at a greater risk for developing a NTD affected pregnancy and advised to consult their doctor regarding the level of supplement to be used (Health Canada, 1998). It is suggested that they take 400 µg of folic acid per day unless planning for a pregnancy. When planning for pregnancy these women are advised to take a daily dose of 4.0 mg from at least one month before conception through the first three months of pregnancy (Spina bifida and Hydrocephalus Association of Canada, 1999; Centres for Disease Control and Prevention, 1992). Also at a greater risk for NTD affected pregnancy are women with diabetes, epilepsy and/or a family history of NTD. These women are also advised to consult their physician for appropriate supplementation (Health Canada, 1998) and may be advised to take 5 mg folic acid per day, before and while pregnant (Motherisk Group, 1999). In India, pregnant women are recommended to consume 400 µg folic acid per day. However, for non-pregnant women over the age of 16 years the recommendation is 100 µg/day (Gopalan et al., 1989). The revision of present recommendations to include a special recommendation for women of childbearing age has been suggested by Krishnaswamy and Madhavan Nair (2001).

As mentioned, the current recommendation for women capable of becoming pregnant is to consume 400 µg SFA/day. Supplements containing folic acid have been shown to effectively reduce the risk of NTD affected pregnancy in a number of clinical trials. Doses in the range of 360 µg SFA/day to 800 µg SFA/day have been used in these trials. However, only 1/4 of women are taking a folic acid supplement (Kloeblen, 1999). Barriers include lack of awareness, lack of motivation and cost. Women of lower socio-
economic status are the least able to afford the long term use of folic acid supplements, although they have a higher probability of giving birth to a NTD affected baby (Kloeblen, 1999).

Due to poor compliance with supplement use, mandatory fortification of grain products with folic acid was introduced in North America in 1998. In Canada, flour, white flour and enriched flour are fortified with folic acid at the level of 150 \( \mu \text{g/100g} \). Precooked rice is fortified at the level of 160 \( \mu \text{g/100g} \). Enriched bread or enriched white bread is fortified at the level of 100 \( \mu \text{g/100g} \). Breakfast cereal, which was fortified prior to 1998, is fortified with folic acid at the level of 60 \( \mu \text{g/100g} \). Alimentary pasta is fortified at the level of 200-270 \( \mu \text{g/100g} \). Fortification of food is a "passive" public health intervention that can increase women's intake of folic acid during the critical embryonic development period (Czeizel, 1998). This is especially important, since it encompasses the entire population including all socioeconomic and ethnic groups (Van Allen, 1994), and also women with unplanned pregnancies. Since it is difficult to educate women on the risk of NTD-affected pregnancies associated with low folate intakes (Rasmussen et al., 1998; Kirke et al., 1992), fortification of foods with folate may be the best means to increase the folate intake of all women of childbearing age. The form of folate used in the fortification of food is the same as that in supplements, and is thus easily utilized by the body. Intake of folate from fortified foods has been shown to be effective in raising red blood cell folate status (Cuskelly et al., 1996).

Folic acid fortification was expected to increase the daily intake of folic acid among women of reproductive age by about 100 \( \mu \text{g/day} \) (Gregory, 1997). Studies in North America have shown this to be true (French et al., 2003; Boushey et al., 2001;
Edmonds et al., 1999). Edmonds et al., (1999) assessed the effect of fortification on dietary folate intake of 289 rural women in the US. They reported that the mean dietary intake of folate in these women increased from 320 µg/day to 489 µg/day. However, the women in this study were between the ages of 18 and 89 years and thus not limited to the women of reproductive age. In another US study, Boushey et al., (2001) estimated that the folic acid fortification increased the mean intake of synthetic folic acid by 180 and 187 µg SFA/day (equal to 300.6 and 312.3 DFE/day, respectively) for women aged 18-39 years and 40-46 years respectively. In Canada, French et al., (2003) estimated that folic acid fortification of bread and grain products contributed 104 ± 68 µg SFA/day (174 ± 114 DFE/day) to the diet of women between the ages of 18 and 45 years in the Vancouver area of British Columbia. Thus, mandatory fortification of bread and grain products in North America meets its expectations of increasing the daily intake of folic acid among women of reproductive age by about 100 µg/day and sometimes almost double. Even so, fortification of grain products by itself does not help women meet the special recommendation of 400 µg SFA/day for reducing the risk of NTD. By combining the daily use of folic acid containing supplements with the fortification program, more women capable of becoming pregnant would be able to meet this special recommendation of 400 µg SFA/day to reduce the risk of NTD.

2.4.2 Folate Intakes of Women of Childbearing age

There is very little information available regarding folate intakes (dietary and synthetic) of Canadians. The last documented comprehensive nutrition survey in British Columbia was conducted in the early 1970's but it did not assess folate intake (Health and
Welfare Canada, 1977). A recent study by Starkey et al., (2001) showed that approximately 50% of women between the ages of 18 and 49 years were not consuming the minimum of five servings of grain products per day. Thus, fortification of grain products might not help all women. Recently, Palaniappan et al., (2001) studied the dietary patterns of smokers and non-smokers in the age group of 18-65 years in Canada. They reported that the mean folate intake of women (n = 970) ranged from 197 ± 112 µg/day smokers to 225 ± 91 µg/day in non-smokers. They also reported that most of these women, irrespective of smoking status, had folate intakes below the EAR of 320 DFE/day. In this study however, the data were collected before the mandatory folic acid fortification of flour began in Canada, and does not give an indication of the present intakes. The recent study by French et al., (2003) is the only report on folate intakes of women of childbearing age living in Lower Mainland British Columbia. This study was done post-fortification. They estimated the mean intake of folate from all sources to be 812 ± 710 µg DFE/day including 314 ± 389 µg SFA (synthetic folic acid)/day. Although approximately 86% women were meeting the EAR, only 27% were meeting the special recommendation of 400 µg SFA/day.

2.4.3 Biochemical Parameters of Folate Status

In order to determine folate adequacy of a population, dietary measures should be combined with biochemical measures of folate. Also, there is evidence that low red blood cell folate and/or high plasma homocysteine may increase the risk of NTD. Red blood cell folate, and plasma (or serum) folate levels are the measures of blood folate status cited in the literature that can be used to assess folate status. Serum or plasma folate
levels reflect short-term changes in folate intakes (during the preceding 1-2 days) in humans. Red blood cell folate on the other hand represents the dietary folate intake during the preceding 120 days (the half life of red blood cell). Red blood cell folate is thus, a more accurate estimation of tissue folate status (Mason, 2003). Red blood cell folate levels ≥ 906 nmol/L (400 ng/mL) is associated with reduced risk of NTD (Daly et al., 1995).

Another important biomarker of folate status is plasma homocysteine level. Ubbink reviewed the role of vitamins in the pathogenesis and treatment of hyperhomocysteinemia, and concluded that a daily supplement of 0.65 mg/day of folic acid can normalize moderate hyperhomocysteinemia (Ubbink, 1997). Brouwer et al., (1999) stated that doses of folic acid as low as 250 µg/day, in addition to the usual dietary folate significantly decreased plasma total homocysteine concentrations in healthy, young women (Brouwer et al., 1999).

Folic acid fortification has had an impact on both plasma folate and homocysteine levels. Schorah et al., (1998) assessed the long-term effect of small increases in dietary folic acid on the concentration of plasma homocysteine in the general population in Leeds, UK. The subjects were randomly assigned to receive un-fortified cereals or cereals fortified with 200 µg of folic acid per portion. Blood samples were taken at baseline, 4, 8, and 24 weeks after treatment. Overall, folic acid fortification of cereals led to significant increases in serum folate (66%; P<0.001) and red blood cell folate (24%; P<0.001), and a decrease in plasma homocysteine (10%; P<0.001). In a randomized, double blind, placebo-controlled, crossover trial, Malinow et al., (1998) showed that plasma folic acid increased and plasma homocysteine levels decreased proportionately with an increased
level of folic acid fortification in breakfast cereal. They studied 75 men and women with coronary artery disease between the ages of 45-85 years and used three different levels of fortification (127, 499 and 655 μg SFA/30 g cereal).

Jacques et al., (1999) assessed the effect of folic acid fortification on plasma folate and total homocysteine concentrations of the Framingham Offspring Study Cohort at the fifth examination (January 1991 to December 1994) for baseline and the sixth examination (January 1995 to August 1998) for follow-up values. They found that the mean plasma folate concentration of the study group (exposed to fortification) who did not use vitamin supplements increased from 4.6 ng/ml (4.3-4.9 ng/ml) to 10.0 ng/ml (9.3-10.7 ng/ml). The prevalence of low plasma folate concentrations (<3 ng/ml) reduced from 22.0% (95% CI: 17.3-26.7) to 1.7% (95% CI: 0.0-5.4). The mean total homocysteine concentration decreased from 10.1 μmol/L (9.8-10.5) to 9.4 μmol/L (9.1-9.7), and the prevalence of high homocysteine concentration (>13 μmol/L) decreased from 18.7% (95% CI: 14.5-22.9) to 9.8% (95% CI: 5.6-14.0). Thus the authors concluded that the fortification of enriched grain products with folic acid was associated with a substantial improvement in the folate status in a population of middle-aged and older adults.

The folate status of 135 healthy non-pregnant women (18-45 years) was investigated by Caudill et al., (2001) in California. This study was conducted post-fortification. They reported an average red blood cell folate level of 1307 nmol/L (± 349) in their subjects, and many (80%) achieved red blood cell folate concentrations ≥906 nmol/L, a level associated with a very low risk of NTD. The total plasma homocysteine level of these women was 5.5 ± 1.7 μmol/L.
What is also of significance is that American cereals are fortified at a much higher level compared to those in Canada. Thus, American women may be consciously or unconsciously consuming higher levels of folic acid and this can be reflected in their blood status. There are limited Canadian data examining the biochemical parameters of folate status of women of childbearing years. The Motherisk group of Toronto recently compared the red blood cell folate levels of women of childbearing age (15-45 years) before and after fortification (Kapur and Koren, 2001). They observed that the red blood cell folate concentration post fortification rose by 80% to a mean of 901 ± 318 nmol/L (p<0.00001) (median 837 nmol/L; range 367 to 2379 nmol/L) from 517 ± 215 nmol/L (median 485 nmol/L; range 132 to 1738 nmol/L) before fortification. They also reported a decrease in the relative risk for NTDs from 2.5 to 1.0 after fortification. Ray et al., (2002), compared the red blood cell folate samples analyzed by MDS Laboratories (Toronto, Ontario, Canada) before and after folate fortification. They reported an increase in the geometric mean red blood cell folate concentration from 527 nmol/L prefortification (January 1, 1996 to December 31, 1997) to 741 nmol/L after January 1, 1998 (mean difference 214 nmol/L). The results from these studies indicate that folic acid fortification has enhanced the folate status of women of childbearing potential and also reduced the relative risk of NTD.

2.4.4 Women’s Awareness and Knowledge of Folate and NTD

Several studies around the world have evaluated women’s knowledge and awareness of folic acid and NTDs, with the premise that if women have an understanding then it would result in behaviour change to use supplements containing folic acid or
consume folic acid fortified foods. This, in turn, could lead to reducing the risk of a NTD affected pregnancy.

**UK and Europe:** Sayers et al., (1997) conducted a community based cross-sectional study with women of childbearing age (15-44 years) in Dublin, Ireland and reported that two thirds of the participants had heard of folic acid and over half of these women correctly reported that it should be taken before becoming pregnant. However only 7% had actually taken folic acid before becoming pregnant. The knowledge of the relationship between folic acid and spina bifida was poor. Approximately 15% of the women had heard of NTDs whereas close to 90% of women had heard of spina bifida. Only 13% of the women who had heard of spina bifida said that a lack of folic acid caused spina bifida and 17% who had heard of folic acid knew that it prevented spina bifida. McGovern et al., (1997) conducted a study to determine use of folic acid supplements and factors affecting their use in 487 women who delivered normal babies in three maternity units in Glasgow, Scotland. They reported that only 21% took folic acid supplements before conception and the lack of awareness of the potential benefits associated with folic acid use was the most common reason cited for not using supplement. Only women who had delivered a healthy child were eligible for participation, and thus, generalizability of these results to all women could be questioned.

A more recent study conducted by Oleary et al., (2001) in Dublin, with women attending antenatal clinics had more encouraging results. Over 90% of the women had heard of folic acid and 67% knew that it could prevent neural tube defects. However, one must keep in mind that these were all pregnant women attending antenatal clinics and thus it is probable that their doctor spoke to them about folic acid and pregnancy. But,
only 30% were advised to take the vitamin periconceptionally and about 18% of the women actually did.

A similar study was conducted in the UK by Sen et al., (2001) with 300 pregnant women in an antenatal clinic. All but two women had heard of folic acid and the majority (91%) knew that folic acid could prevent neural tube defects. Seventy-six (76%) percent knew the correct timing of taking folic acid and close to a half (134/300, 44.6%) of the women reported having taken folic acid in the periconceptional period. Since the participants of this study were already pregnant, it is possible that they had already discussed folic acid with their doctors and thus, generalizibility of these results to other women of childbearing potential could be questioned. Folic acid promotional campaigns in Ireland and UK may also have contributed to the increased awareness.

de Jong-van den Berg et al., (1998) evaluated the impact of non-systematic information on periconceptional folic acid use. The proportion of women who had heard of folic acid prior to the Dutch Ministry of Health advising any women planning a pregnancy to take 0.5 mg of folic acid daily increased from 28% to 78% one year after the recommendation. Even though the proportion of women that took folic acid supplements during the recommended period also increased from 0.8% to 4.4%, still, it represented a small fraction of the targeted population.

Australia: National and statewide education campaigns in Australia have been successful in increasing the knowledge and awareness of women regarding folic acid and its significance. A statewide health promotion project was undertaken in Western Australia from mid 1992 until March 1995, in order to inform women about folate and spina bifida and to encourage women to increase their folate intake (Bower et al., 1997). The
campaign consisted of posters, pamphlets and information sheets mailed to general practitioners, pharmacists and other health professionals. For the public campaign paid and unpaid media items were launched in November 1992. Following the campaign, the proportion of women who knew the relationship between folic acid and spina bifida increased from 8% to 68%. However, only $\frac{1}{3}$rd took a folic acid containing supplement before and in early pregnancy. It is evident from this result that the increase in awareness does not necessarily translate into a change in behaviour.

Chan et al., (2001) who recently evaluated a similar campaign in South Australia also reported a significant increase in the knowledge of folate and the proportion of women taking periconceptional folic acid supplements. The proportion of women who knew that sufficient folic acid/folate in a mother’s diet may prevent spina bifida and the correct timing of increasing the folate/folic acid intake to prevent spina bifida increased from 26% and 12% respectively before the campaign in 1994 to 42% and 29% respectively after the campaign in 1995. These authors also found that the sales of supplements containing 0.5 mg folic acid in South Australia doubled after the campaign (and increased by another 10% a year later) and the total prevalence of neural tube defects declined between 1966 and 1999 from a baseline of 2.0 per 1000 births to 1.1 per 1000 births (Poisson regression, $p=0.03$; average decline of 1.0% per year).

United States: In 1995, prior to a public awareness campaign in the US, only 52% women had heard of folate. Five percent of women knew folic acid prevented birth defects and only 2% knew that folic acid should be taken periconceptionally to reduce the risk of NTD. Recently, after a public awareness campaign, the March of Dimes Birth Defects Foundation surveyed women between the ages of 18-45 years and found that there was an
increase in women having heard of folic acid (68%), women knowing that folic acid helped prevent birth defects (13%), and women knowing that folic acid should be taken before conception (7%) (Centres for Disease Control and Prevention, 1999).

Canada: There have been few studies assessing the level of awareness and knowledge of folic acid among Canadian women. Bonin et al., (1998) examined the level of knowledge about the usefulness of periconceptional folic acid supplementation in a cross-sectional survey of 1124 women between 16 and 40 years of age in twenty-two Canadian teaching practices affiliated with the Northeastern Ontario Primary Care Research Group. They found that more than half of the women (63%) were familiar with NTDs but only 8% women knew the direct link between supplementation with folic acid and the prevention of NTDs and only 2% were aware of the periconceptional requirement for folic acid supplementation. In another study conducted by Neimanis et al., (1999) with 484 women who delivered healthy babies, 63% women were aware of the need for periconceptional folic acid however only 34% of the respondents actually reported taking vitamins before conception. Similarly, a high level of awareness was found in another hospital based study conducted with pregnant women and women in the postpartum period in British Columbia (Morin et al., 2001). Seventy-one percent knew that vitamins could prevent birth defects. Of these women, 76% identified folic acid as the one vitamin specifically associated with the reduction of birth defects. About half of the women (49%) actually took vitamins prior to pregnancy. In both of the above mentioned studies (Neimanis et al., 1999; Morin et al., 2001) the higher level of awareness could be attributed to the fact that these women were either pregnant or in the postpartum period and probably had discussed the issue with their doctor. French et al., (2003) conducted a cross-sectional
study with 148 non-pregnant women of childbearing age (18-45 years) in the Lower Mainland, British Columbia. Most of the participants (95%) in their study had heard of folate, however only one-fourth was aware that folate could prevent birth defects. One reason for a lower awareness among Canadian women compared to the Australian women is that Australia has had more aggressive public education campaigns. In March 2002 Health Canada launched a public education campaign to increase the level of awareness of folic acid and NTDs among Canadian women by targeting doctors and other health practitioners. The results of this strategy have not been evaluated yet.
2.5 Background on Dietary Habits and Folate Status of Sikh Women

2.5.1 Dietary Habits of Sikh People

The Sikh people are a religious sect of the East Indian population, originally from the northwestern State of Punjab in India (Figure 3) (Baird, 1983; Keena et al., 1987). They are believed to be the descendants of the Aryan tribes that entered India from the northwest about 1500 BC and settled there (Baird, 1983; Keena et al., 1987). They are followers of Sikhism, a religion that was founded by Guru Nanak Saheb about 500 years ago. Sikhism is considered to have emerged from Hinduism, sharing with it major concepts such as reincarnation while rejecting others such as vegetarianism. Even though the Sikhs are not strict vegetarians they tend to consume a predominantly vegetarian diet (Baird, 1983; Keena et al., 1987; Drakulic and Tanaka, 1981). Wheat is their staple crop and 'roti', a flat thin crepe made with whole-wheat flour, is their staple food. Milk and milk products are commonly used and traditionally beef is not eaten (Drakulic and Tanaka, 1981). The Sikhs eat 'Khatka meat', which means the animal is killed with one blow and not bled (Carlson et al., 1984). They generally tend to consume a high fiber diet derived from roti/chapatis, pulses, and fruits (Carlson et al., 1984). In a study conducted in the UK, Punjabi Sikhs and Hindus were found to eat dhal (lentils) more often compared to other south Asians (Simmons and Williams, 1997). Besides adhering to their traditional dietary patterns, as the Sikh people migrate to more western societies like England and North America there is also the addition of "Western foods" such as corn flakes, bread (with butter as the preferred fat), fast foods and convenience foods (Baird,
1983; Wharton et al., 1984). Compared to the white population Sikhs tend to drink less coffee, but tea with cream and sugar is common (Drakulic and Tanaka, 1981).

Cooking involves two basic steps (Sekhon, 1996). The first step is preparing the 'tarka' using a combination of sautéing and braising. 'Tarka' is when a variety of spices are added to frying onions. In the second step the main ingredients are added to the onions (vegetables are added to make a sabji, meat to make a curry, and cooked lentils to make a dhal). These are then cooked for a prolonged period of time. South Asian curry is a spiced dish with a thick sauce and does not necessarily contain curry powder. Instead, a mixture of freshly ground seasonings called 'garam masala' is added to the curries. Deep-frying and pan-frying appear to be common cooking methods amongst this population, especially for snacks and appetizers. A typical meal consists of roti/rice, sabji, dhal or meat curry with yogurt or raita (contains stirred yogurt mixed with vegetables like onions, tomatoes, carrots, cooked squash, or chick pea flour dumplings). Evening meals still tend to be more traditional, compared to breakfast and lunch, which may be more westernized (Sekhon, 1996).

2.5.2 Folate Intake of Sikh Women

The Sikh population is a high-risk population for having NTD affected pregnancies. They have demonstrated an occurrence rate of 3.87:1000 live births (Hall et al., 1988; Baird, 1983; Chambers et al., 1994), which is similar to the highest provincial rates found in Canada, namely Newfoundland and Quebec. The two possible factors contributing to a high risk in this population are genetic predisposition and environmental factors such as diet (Turner and McCourt, 1998).
There is limited information available regarding the folate intake of the Sikh population. In the literature, there was only one study from the UK that actually studied the folate intake of Sikh women. Sikh women tend to eat a predominantly vegetarian diet, with green leafy vegetables and lentils, which are good sources of folate. In a hospital-based study conducted in Birmingham, UK (1984), the mean dietary intake of folate by Sikh pregnant women was 131 μg/day (57-389 μg/day) (Wharton et al., 1984). In another study comparing the intakes of Indian and Caucasian vegetarians with Caucasian omnivores, the mean intake of dietary folate for the Indian vegetarian women was 142 μg/day (SEM = 10.2), lower than the Caucasian vegetarians (262 μg/day) and Caucasian omnivores (169.6 μg/day) (Reddy and Sanders, 1990).

2.5.3 Biochemical Indicators of Folate Status in Sikh Women

As with diet, literature is scarce on biochemical data for folate status of Sikh women. Michie et al., (1998) reported that the red blood cell folate concentration of women of Indian and Pakistani origin in the North Thames (West) Region of the UK tended to be lower [246 ± 87 ng/ml] than that of white women [286 ± 160 ng/ml (P<0.02)]. Sikh women who tend to consume a predominantly vegetarian diet, high in folate, had red blood cell folate levels that tended to be lower. Perhaps the lower red blood cell folate values could be due to: 1) Dietary folates have not been proven to be as effective in improving blood folate status, as is synthetic folic acid; 2) Sikh people may overcook some of their traditional recipes, and since folate is heat sensitive and water soluble, it could be lost in the process of food preparation.
2.5.4 Sikh Women’s Knowledge of Folate and NTD

To date there are no studies that have assessed the knowledge and awareness of folate and NTD in Sikh women, in India or around the world. There is the only one published study on the knowledge of folic acid and NTDs among women of childbearing age in India. Gupta and Gupta (2000) conducted a survey of 50 well-educated married women of childbearing age belonging to the upper middle class in New Delhi (neighbouring city to the state of Punjab), India. Only 10 women had heard of folate and they acquired this information during the second or third trimester of pregnancy as they were prescribed folic acid along with iron for prevention of anemia. None of these women knew of a link between folic acid and NTDs.

In Vancouver, BC, Morin et al., (2001) conducted a hospital-based survey of pregnant or postpartum women, which included 50 East Indian women. Twenty percent of the East Indian women knew that vitamins could help prevent some birth defects and 48% of the women reported that they used vitamins prior to conception. The authors have not defined the term ‘East Indian’, so it is not known if the sample included any Sikh women. Also, this study was done in English and thus would have missed out on East Indian women that do not read or write English fluently. French et al., (2003) recently assessed knowledge and awareness of folic acid and neural tube defects in women of childbearing age in the Lower Mainland, British Columbia. Their sample consisted of predominantly white women and thus the results may not be applicable to Sikh women. Despite the fact that Sikh women are at a very high risk of having a NTD affected pregnancy, we still lack documentation on the knowledge and awareness of these women about the association of folic acid and NTD risk.
2.5.5 Sikh Women’s Beliefs and Perceptions Regarding Diet During Pregnancy

During pregnancy, certain food taboos may be followed by Sikh women, which could affect their folate status. Keena et al., (1987), suggested that during pregnancy Sikh women’s diet may be restricted to avoid the fetus from getting too large and having a difficult delivery. This practice of "eating down" during pregnancy is common in India. Women do not believe that eating less food will harm the fetus (Choudhry, 1997).

Based on traditional Hindu (Ayurvedic) beliefs, food substances are recognized as having 'hot' and 'cold' qualities, which relate to the effects they have on the body rather than the temperature or taste of the food itself (Choudhry, 1997). Because pregnancy generates a state of hotness, it is thought to be desirable to attain balance by eating cold foods. Cold foods such as milk, wheat, and/or green leafy vegetables, are considered beneficial and as a result recommended during the early pregnancy. Hot foods such as beans, pulses, meat, fish, eggs, eggplant, and/or papaya, are considered harmful and thus avoided during the earlier part of the pregnancy. However the hot foods are encouraged during latter stages of pregnancy to facilitate the expulsion of the fetus. The concept of hot and cold varies from region to region. We do not know if the Sikh women in the Lower Mainland, BC believe hot/cold foods and if they do, what foods they consider as being hot or cold.
2.6 Summary

There is documented data showing that the Sikhs demonstrate a high risk of spina bifida, however we lack evidence about the folate status of Sikh women of childbearing age in Canada and elsewhere. Thus, the overall purpose of this study was to examine the folate status of Sikh women of childbearing age living in the Lower Mainland, BC. Specific objectives of this study were:

1. To determine the folate status of this group:
   - By determining the dietary folate intake and
   - By determining red blood cell folate concentration and blood homocysteine levels

3. To study the knowledge of the participants regarding the relationship between folate and NTD.

4. To determine the beliefs, attitudes and perceptions of these women regarding diet and/or nutrition during pregnancy.
CHAPTER 3: PROJECT RATIONALE AND OBJECTIVES

3.1 Introduction

The overall purpose of this study was to assess the folate status of Sikh women of childbearing age living in the Lower Mainland, BC. We were also interested in studying the knowledge of these women regarding the relationship between folic acid and NTD, and gaining an understanding of their beliefs and attitudes towards diet and nutrition during pregnancy. The specific objectives were to determine:

1. Folate intakes of Sikh women of childbearing age, living in the Lower Mainland, BC.
2. Red blood cell folate levels of Sikh women of childbearing age.
3. Plasma homocysteine levels of Sikh women of childbearing age.
4. Sikh women's knowledge level and attitude towards the relationship between folate and NTD risk.
5. The beliefs and perceptions of Sikh women regarding diet and nutrition during pregnancy.

The following sections will briefly state the rationale for studying these objectives and research questions addressed for each objective.

Objective 1:

To determine folate intakes of Sikh women of childbearing age, residing in the Lower Mainland, BC.
**Rationale:** Inadequate folate status has been associated with NTD risk and synthetic folic acid intake has shown to reduce the recurrence and occurrence of NTD. In the 1990’s recommendations for women of childbearing age were revised to 400 µg/day of synthetic folic acid consumption. To help women achieve this goal, grains and flour are now fortified with folic acid in Canada. Although there is evidence that the Sikh population is at high risk for NTD affected pregnancies, there is a lack of information regarding the dietary folate intake of Sikh women of childbearing age. A recent study by French et al., (2003), examined dietary folate intakes of women living in the lower mainland of BC, but their population sample comprised of all English speaking and predominantly Caucasian women. Sikh women consume ethnic and traditional food items besides the average Canadian diet. Thus, assessing folate intakes of Sikh women of childbearing age in BC, will help determine if this population is meeting the special recommendation of folic acid for women capable of becoming pregnant, to reduce NTD risk.

**Research Questions:**

1. What is the daily folate intake of Sikh women of childbearing age living in the Lower Mainland, as assessed by 7 multiple 24-hour dietary recalls over a period of four weeks?
2. How does folate fortification of flour and grain products contribute to Sikh women's folate intake?
3. How does the use of folic acid containing supplements contribute to Sikh women's daily folate intake?
4. What food items included in the diet of Sikh women are rich/good sources of folate?
5. Which foods that are not traditionally classified as good sources of folate are consumed in frequencies high enough to contribute substantially to the folate intake of Sikh women?

6. What is the contribution of traditional or ethnic foods consumed by Sikh women, to the overall folate intake of Sikh women?

**Objective 2:**

To determine red blood cell folate levels of Sikh women of childbearing age, living in the Lower Mainland, BC.

**Rationale:** Red blood cell folate is an indicator of folate status of an individual and has been linked to the risk of NTD-affected pregnancy. Daly et al., (1995) showed that red blood cell folate levels of 400 µg/mL (906 nmol/L) or more were associated with reduced NTD risk. To date there is no study that has looked at the red blood cell folate levels of Sikh women in Canada or elsewhere.

**Research Questions:**

1. What is the mean red blood cell folate concentration of Sikh women living in Lower Mainland, BC?

2. What percentage of Sikh women has a red blood cell folate concentration below 906 nmol/L?

3. Do the daily folate intakes of Sikh women relate to the red blood cell folate concentrations?
Objective 3: To determine the plasma homocysteine levels of Sikh women of childbearing age, living in the Lower Mainland, BC.

Rationale: Selhub and colleagues (1993) indicated that plasma homocysteine is a sensitive marker for cellular folate status and a number of studies have used it in assessing folate status (Jacques et al., 1999; Caudill et al., 2001; Ray et al., 2001). Hyperhomocysteinemia has recently been linked to increased risk of NTD-affected pregnancy via folate-homocysteine metabolism (Figure 5) (Rosenquist et al., 1996; Epeldequi et al., 2002). We, however, lack information on homocysteine levels of Sikh women in Canada or elsewhere.

Research Questions:

1. What is the mean plasma homocysteine level of Sikh women of childbearing age living in Lower Mainland, BC?

2. What percentage of Sikh women has a plasma homocysteine level greater than 7.8 \( \mu \text{mol/L} \)?

3. Does the daily folate intakes of Sikh women relate to their plasma homocysteine levels?

4. Does the red blood cell folate levels of Sikh women relate to their plasma homocysteine levels?

Objective 4: To determine the knowledge level of Sikh women regarding the relationship between risk of NTD-affected pregnancy and folate status.
**Rationale:** Knowledge and awareness of the relationship between folic acid and NTDs among women of childbearing age has been evaluated by a number of studies all over the world including Canada, the US, Europe, Asia and Australia. However, to date we lack information on the knowledge of Sikh women regarding this relationship. There is no data available regarding the potential sources of information on folic acid and NTD or the role the family physician plays in imparting this information to these women.

**Research Questions:**

1. What percentage of Sikh women of childbearing age living in the Lower Mainland, BC, is aware of the relationship between folic acid and NTD?
2. What percentage of Sikh women are currently consuming a folic acid containing vitamin?
3. What percentage of Sikh women would be willing to take daily folic acid supplements, if they were aware of the relationship between folic acid supplement intake and the reduction in the risk of a NTD-affected birth?
4. What percentage of Sikh women can identify foods that are good sources of folate?
5. Are Sikh women aware of folate fortification of bread and grains?
6. Where do Sikh women receive their nutrition information?

**Objective 5:**

To study the beliefs and perceptions regarding diet and nutrition during pregnancy of Sikh women living in the Lower Mainland, BC.
**Rationale:** The review of the literature has revealed that South Asian Indian women tend to believe in certain 'Food Taboos' and as a result avoid certain foods during pregnancy (Mahat, 1998; Narayan, 1997; Keena et al., 1988). 'Food Taboos' vary from region to region. We lack information on how Sikh women in BC perceive the role of diet during their pregnancies, what is their definition of a healthy diet for pregnancy and how this may affect their nutrient intakes, specifically folic acid.

**Research Questions:**

1. Do Sikh women believe in any 'food taboos'? If yes, what are they? Where did they learn about them?

2. Would these women avoid any foods during pregnancy? If yes, which ones and why?

3. Would these women add any foods to their diet during pregnancy, or increase the intake of any foods during pregnancy? If yes, which ones and why?

4. Are there foods that may be avoided/added during any particular period of pregnancy? If yes, why?

5. How would these taboos affect folic acid intake of Sikh women?
CHAPTER 4: EXPERIMENTAL DESIGN AND METHODOLOGY

4.1 Introduction

The work described in this project was funded by the Spina Bifida and Hydrocephalus Association of British Columbia. Ethical approval was received from the University of British Columbia Office of Research – Ethical Reviews (Appendix 1).

The present study involved descriptive quantitative research methods to provide a statistical profile of the sample population. A convenience sample of volunteer Sikh women who were English or Punjabi speaking, between the ages of 18 and 45 years of age, participated. Inclusion criteria included non-pregnant and/or non-lactating at the time of the study and for the previous six months and living in the Lower Mainland, BC. Data were collected through individual interviews and blood tests. The interviews involved a verbally administered survey designed to describe and quantify the knowledge and behaviors of these women regarding folate and its relationship with NTD. The dietary data were collected through seven 24-hour dietary recalls. The first recall was conducted in person and the remaining six by telephone calls placed during the subsequent four weeks. These data were analyzed to assess the folate intake of the sample population. Blood tests were performed by BC Biomedical Laboratories, to assess the red blood cell folate and plasma homocysteine levels.
4.2 Methodology

In this section subject recruitment, the procedures involved in the collection and analysis of folate intake data, biochemical parameters of folate status and the knowledge and behaviours of Sikh women will be discussed.

4.2.1 Subjects

4.2.1.1 Sample Size

Since our study population is fairly homogeneous, the sample size of 33 women was considered to be sufficient to achieve the study objectives through consultation with a statistician and as indicated in the literature (Thompson and Byers, 1994). To account for dropouts from the study during the follow-up period, a total of 50 women were recruited.

4.2.1.2 Inclusion/Exclusion Criteria

All Sikh women who were between the ages of 18 and 45 years, fluent in English and/or Punjabi and residing in the Lower Mainland were eligible to participate in the study. A Sikh woman was defined as being Sikh if she was born to a Sikh family, i.e., both her parents were Sikh by birth and could trace their family roots to Punjab, India. Women that had been pregnant or lactating in the past six months were excluded from the study because pregnancy and lactation could alter regular eating habits and also there are quite a few food taboos surrounding pregnancy and lactation among Punjabi women that could impact intake.
4.2.1.3 Recruitment of Participants:

Four routes were used to recruit women for the study. First, posters were placed in areas that were visited frequently by Sikh women, e.g. the Punjabi market on Main Street in Vancouver and Scott Road in Surrey/Delta. As well, posters were placed in institutes that attend to Sikh women’s health or social needs, such as the Progressive Inter-Cultural Society (PICS) and the Options Community Services in Surrey. The institutes were contacted by a letter describing the purpose of the study (Appendix 2), and with their permission, a recruitment poster/flyer (Appendix 3) was posted. The poster briefly explained the purpose of the study without indicating folic acid as the nutrient of interest. Secondly, an advertisement was placed in a local free Punjabi newspaper. Third, participants were recruited by setting up an information booth in the staff room of Khalsa Public School in Surrey. Lastly, due to a low response rate by the above means of recruitment, women were recruited by word of mouth. Women who were interested in participating submitted their name, phone number and a convenient time to reach them. They were then contacted by phone to briefly explain the purpose of the study. If the woman was eligible and still interested in participating in the study, an in-person interview was arranged at that time. Women agreeing to participate in the study were also asked to talk to their friends and family about the study in search for more participants. Women agreeing to participate in the study were informed about the purpose of the study, and written informed consent (Appendix 4) was obtained from the women prior to beginning the in-person interview.
4.2.2 Data Collection

The total process of data collection spanned over a four-month period from mid April 2001 to August 2001 and included obtaining folate intake data and blood work for the assessment of red blood cell folate and plasma homocysteine. As well, data were collected to assess the women's knowledge and attitudes regarding folic acid and neural tube defects and their perceptions regarding diet during pregnancy.

4.2.2.1 Folate intake

4.2.2.1a Developing and Validating a Food Frequency Questionnaire (FFQ)

The use of a culturally relevant FFQ was initially proposed in order to reduce the respondent burden in determining the folate intake. Detailed discussion of the development and validation of the FFQ are presented in Appendix 5 and the FFQ itself is presented in Appendix 7.

The FFQ overestimated the folate intake compared to the 7-day dietary records. Over reporting of the intake of food items was observed in FFQ compared to the 7-day dietary records. For example, one woman reported consuming two oranges per day in her FFQ while her dietary records added up to only about half an orange per day. Another woman reported consuming one cup of lentils per day in her FFQ compared to only one fifth of a cup in her dietary records. Herbert et al., (1998 and 1999) have reported similar results to ours in their attempts at developing a FFQ for use with people of Gujarat and Kerala, India. They attributed the over estimation of nutrient intake by FFQ to social biases namely, social desirability or social approval.
4.2.2.1.b Seven-day Dietary Recalls:

Since we were unable to validate the FFQ, it was decided to assess the targeted population’s folate intake using seven 24-hour dietary recalls. In an attempt to accommodate the women that were not fluent in English and the inability of the researcher to read and write Punjabi, seven 24-hour dietary recalls were conducted instead of having the participants self-report their 7-day dietary records. The first 24-hour dietary recall was conducted at the time of the first in-person interview, which lasted for approximately 20 minutes. The participants were asked for the most convenient time of day for the researcher to telephone over the subsequent four weeks to conduct the six remaining dietary recalls. The women were not aware of the day that the researcher would contact them to conduct these recalls. Representative number of week days and weekend days were included. The 24-hour dietary recalls of the previous days’ food and beverage consumption were obtained using the multiple pass method developed by the US Department of Agriculture (USDA) for use in food intake studies (USDA, 1997). The multiple pass method involves three ‘passes’ to obtain information on the food and beverage intake of the previous day. In the first pass the respondent was asked to recall everything eaten the previous day. The second pass asked for the description and further information (e.g. brand names, spices/condiments used, ingredients of a mixed dish) about the foods mentioned in the quick list. Finally in the third pass all the items recorded in the previous two passes were reviewed with the respondent, and also the respondent was probed for any additional foods eaten and to clarify portion sizes. Since it was not possible to use food models for estimating portion sizes during the telephone interviews,
common household measures (such as measuring spoons, cups, etc) were used whenever necessary. Each telephone interview was completed in approximately 15 minutes.

4.2.2.2 Knowledge and Behaviors Questionnaire

4.2.2.2a Development of the Women's Knowledge and Behaviors Questionnaire

A survey was developed to determine Sikh women's knowledge of the relationship between folate intake and NTD risk and to understand their beliefs and perceptions toward diet and folic acid supplementation. A preliminary (Appendix 8) survey was developed using the knowledge and behaviors questionnaire developed by French et al., (2003) as a guideline. The first part of the questionnaire included questions regarding the following: knowledge of food sources of folate; the association of folate with NTD and the sources of this information; attitudes toward the use of folic acid supplements; knowledge of folic acid fortification and sources of such information; the participants' perception of the role of diet during pregnancy; any changes that they might make to their diet during pregnancy and the concept of 'hot' and 'cold' foods that is prevalent in the Indian population. The second part of the questionnaire collected demographic information including age, socioeconomic status, education, and length of residency in Canada, current consumption of folic acid supplements, smoking, consumption of alcohol, dietary restrictions and grocery shopping practices.

Once a preliminary questionnaire was developed, focus group meetings were conducted with two groups of Sikh women, to ensure that the questions included in this questionnaire were phrased appropriately for the target group and would be effective in
obtaining the desired information. The women participating in each focus group had characteristics similar to the target population. These women were recruited from the Progressive Inter-Cultural Society and the Options Community Services in Surrey, two organizations catering to the needs of Sikh women in the Lower Mainland, BC. The institutes were contacted by a letter (Appendix 9) describing the purpose of the study. The meetings were held at these institutes and written informed consent (Appendix 10) was obtained from the women before beginning the focus group discussions. These discussions focused on the relationship between NTD and folate, beliefs and attitudes surrounding food and diet, and preferred sources of nutrition related information. As stated, the preliminary questionnaire developed was used as a guide (Appendix 8) to facilitate these discussions. Potential survey questions were posed to these women and their responses were manually recorded. Data collected from the focus group discussions were used to finalize the questionnaire. These discussions were also used to identify ethnic foods consumed by this group that were good sources of folate in their diet. The food frequency questionnaire developed by French et al., (2001) was used as a guide in this case. The final questionnaire (Appendix 11) developed was pilot tested for ease of administration and appropriate phrasing of the survey questions during an in-person interview with two Sikh women, one fluent in English and the other in Punjabi, to ensure effective verbal translation of the questions into Punjabi.

4.2.2.2b Administration of Knowledge and Behaviors Questionnaire

The knowledge and behaviors questionnaire was verbally administered during the second in-person interview, which was conducted following the six telephone 24-hour
dietary recall interviews. At this time they were asked about their current consumption of nutritional supplements and the folic acid content of the supplement consumed by the woman was recorded. Each interview lasted for approximately 30 minutes.

4.2.2.3 Blood Collection

An arrangement was made with the BC Biomedical Laboratories, for blood analysis of red blood cell folate and plasma homocysteine. Our study was given a unique account number. Requisitions (Appendix 12) for blood tests were given to the women after the administration of the knowledge and behaviors questionnaire. Women were asked to go to the BC Biomedicals Laboratory closest to their home. BC Biomedical Laboratories collected and analyzed the blood sample for red blood cell folate and plasma homocysteine levels for each participant.

4.2.3 Data Analysis

4.2.3.1 Analysis of Folate Intake

The folate intake was examined as:

- Dietary intake only (natural folate + fortification)
- Dietary intake without fortification
- Dietary intake (with fortification) and supplemental intake
- Daily folic acid intake from supplemental forms

Folate intake was expressed as μg synthetic folic acid (SFA), Dietary Folate Equivalents (DFE) and/or total folate (SFA + natural folate), as and where applicable. Synthetic folic acid intake was determined by the amount of folic acid contributed by
fortified bread and grain products, fortified ready to eat cereals and folic acid containing supplements. Dietary Folate Equivalents were calculated by multiplying the contribution of SFA by a factor of 1.67, thus adjusting for the greater bioavailability of SFA (SFA is 1.67 times more bioavailable than natural folate, i.e. 1µg SFA = 1.67 DFE).

4.2.3.1.a Estimation of Daily Folate Intake

The dietary intakes provided by the seven 24-hour dietary recalls were entered into Food Processor II Program (Version 7.22, ESHA Research, Salem, Oregon). This computer program contains values obtained from measurements of the total folacin content in foods and includes all compounds related to folate. Canadian and American products are both fortified with folic acid, but this ESHA database had not been updated to reflect the fortification of Canadian food products following the 1998 folic acid fortification. Therefore the folate values for food items affected by the mandatory folic acid fortification program were obtained from the American database of ESHA, which had been updated and similar to the levels of fortification in Canada. Since the level of fortification of ready to eat cereal was unchanged with the 1998 fortification of folic acid, Canadian values were used for these. The results obtained from the analysis of the seven 24-hour dietary recalls were used to describe the usual folate intake of the population and to rank individuals according to high and low folate intake.

4.2.3.1.b Effect of Folic Acid Fortification

To estimate the effect of the 1998 folic acid fortification program on the folate intake of Sikh women, the amount of SFA for most items fortified with folic acid was
adapted from the Canadian Nutrient File 2001 (Health Canada, 2001). The Canadian Nutrient File 2001 did not have the SFA content for all food items used. For such food items the SFA was obtained from the USDA data. This gave us the SFA content of all food items needed to analyse the diets of our population, with the exception of 'roti' and other ethnic food items made from special roti flour. In this case the actual level of fortification of the flour as reported by the manufacturer was used.

4.2.3.1.c Contribution of Folic Acid Containing Supplements

On the knowledge and behaviors questionnaire, participants were asked to report how often they took a supplement containing folic acid. The interviewer confirmed the dose of folic acid provided by the supplement. The average intake of SFA from supplement was calculated based on the folic acid content of the supplement and the frequency of consumption. For example, if a participant took a supplement containing 400 µg of folic acid four times a week, the daily intake of SFA from supplement would be calculated by multiplying the dose (400µg) by the frequency of use (4 days) and dividing the product by the time period covered (7 days), resulting in an average intake of 228.6 µg SFA from supplement per day. The mean daily folic acid intake from supplements was calculated only for the women considered regular supplement users (i.e., women who use folic acid containing supplements at least once per week).

4.2.3.1.d Major Contributors of Folate to the Diet

Once all the records were entered into the Food Processor II Program, food lists for all the participants were combined into one list. The folate contribution of each food
item in this list was summed and the top ten dietary sources of folate in the study population were identified and ranked in their order of contribution to total folate intake. As well, all the ethnic food items from each food list were combined into one comprehensive ethnic food list and these foods were ranked in their order of contribution to total folate intake.

4.2.3.2 Analysis of Questionnaire Data

The Knowledge and Attitudes Questionnaire was used to collect data on Sikh women’s knowledge of folic acid and NTDs and their attitudes towards diet and nutrition during pregnancy. Frequencies regarding the responses to the questions in the questionnaire were determined to understand the population better.

4.2.3.2.a Identification of Good Sources of Folate

Women were provided with a list of various foods and asked to indicate what foods they thought were good sources of folate by indicating “yes”, “no” or “don’t know”. Scores were assigned to each response. For each food item that was correctly identified as a good source of folate or not a good source of folate, a score of +1 was assigned, and a score of −1 was assigned to each food item incorrectly identified. A “don’t know” response was assigned the score of 0. The scores for each of the nine food items included in this question were summed and the total was used to rank women’s knowledge of foods that are good sources of folate. The maximum score possible was 9 and scores were ranked as follows: 7-9 = “very good”, 4-6 = “good”, 0-3 = “poor” and <0 = “very poor”. Scores of this question were compared with the woman’s folate intake to
determine if there was a correlation between knowledge of folate rich foods and dietary intake of folate.

4.2.3.2.b Awareness of Folic Acid Fortification

Women were provided with a list of various foods and asked to indicate whether they thought the food had been fortified with folic acid. The number of correct responses provided a measure of the women’s awareness of folic acid fortification. Scores were assigned to each response. For each food item that was correctly identified a product fortified with folic acid or not, a score of +1 was assigned, and a score of −1 was assigned to each food item incorrectly identified. A “don’t know” response was assigned the score of 0. The scores for each of the seven food items included in this question were summed and the total was used to rank women’s knowledge of foods that are good sources of folate. The maximum score possible was 7 and scores were ranked as follows: 4-7 = “very good”, 3-4 = “good”, 0-2 = “poor” and <0 = “very poor”. Scores of this question were compared with the woman’s folate intake to determine if there was a correlation between knowledge of folic acid fortification and dietary intake of folate.

4.2.3.3 Blood Folate and Homocysteine Analysis

Red blood cell folate levels were measured using a competitive immunoassay, direct chemiluminescent technology, with the ADVIA® Centaur™ System (Appendix 13). The plasma homocysteine levels were measured using an enzyme immunoassay as outlined in Appendix 14.
4.2.4 Statistical Analysis

The raw data were statistically analyzed using programs available in the Statistical Package for the Social Sciences (SPSS, Version 8.0, Chicago, IL). Descriptive statistics such as mean, median and frequencies were calculated for all of the raw data. Correlations between responses on the knowledge and behavior questionnaire and folate intakes were examined to determine if any relationship existed between various survey responses and intake (e.g., if there was a correlation between knowledge of food sources of folate and folate intake). Correlations were also examined between folate intake and RBC folate concentration and plasma homocysteine levels to determine if a relationship existed between them. A regression analysis was done to identify trends associated with different variables such as RBC folate, plasma homocysteine, dietary folate intake and synthetic folic acid intake. Chi square analysis was conducted to determine if there were any associations between categorical variables such as knowledge of folate and age, income and education. Variables examined in Chi square analysis included marital status, education level, age group, income level, years in Canada, previous pregnancy or childbirth, year of youngest child’s birth, and language fluency. All tests were conducted at the p<0.05 level of significance.
CHAPTER 5: RESULTS

5.1 Subjects

5.1.1 Compliance

Fifty women were recruited for the study. Out of this 50, 45 women completed the seven 24-hour dietary recalls and participated in the Knowledge and Behaviors questionnaire. Two women did not complete the seven 24-hour dietary recalls and three did not complete the Knowledge and Behaviors questionnaire. Of the 45 women that completed the dietary recalls and the questionnaire, four did not go for the blood tests. Thus a total of 41 women completed all parts of the study and 45 completed all but the blood tests.

5.1.2 Demographic Characteristics

Table 1 depicts the demographic data obtained from the 45 participants. The majority of the participants had completed university or college, lived in Canada for six years or more, were married, had children and an annual household income less than or equal to $40,000. The median year of the most recent childbirth was 1996 and very few women had experienced miscarriage (n=3) or had a child with a birth defect (n=4). None of the reported defects appeared to be related to a folate deficiency.
TABLE 1: Demographic Characteristics of the Study Participants (n=45) Expressed as Raw Numbers and Percentages where Applicable.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in Years (mean ± SD)</td>
<td>32.2 ± 6.9 (range; 20-45 years)</td>
</tr>
<tr>
<td>Education Level:</td>
<td></td>
</tr>
<tr>
<td>Less than High School</td>
<td>7 (16%)</td>
</tr>
<tr>
<td>Completed High School</td>
<td>11 (24%)</td>
</tr>
<tr>
<td>Completed University or College</td>
<td>27 (60%)</td>
</tr>
<tr>
<td>Years in Canada:</td>
<td></td>
</tr>
<tr>
<td>Less than 1 year</td>
<td>5 (11%)</td>
</tr>
<tr>
<td>1 year – 5 years</td>
<td>6 (13%)</td>
</tr>
<tr>
<td>6 years – 10 years</td>
<td>16 (36%)</td>
</tr>
<tr>
<td>Greater than 10 years</td>
<td>18 (40%)</td>
</tr>
<tr>
<td>Household income:</td>
<td></td>
</tr>
<tr>
<td>&lt;$20,000</td>
<td>8 (18%)</td>
</tr>
<tr>
<td>$21,000-30,000</td>
<td>10 (22%)</td>
</tr>
<tr>
<td>$31,000-40,000</td>
<td>11 (24%)</td>
</tr>
<tr>
<td>$41,000-50,000</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>$51,000-60,000</td>
<td>6 (13%)</td>
</tr>
<tr>
<td>&gt;$60,000</td>
<td>6 (13%)</td>
</tr>
<tr>
<td>Marital Status:</td>
<td></td>
</tr>
<tr>
<td>Single/divorced</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>Married</td>
<td>36 (80%)</td>
</tr>
<tr>
<td>Have Children:</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>34 (76%)</td>
</tr>
<tr>
<td>No</td>
<td>11 (24%)</td>
</tr>
<tr>
<td>Year of Last Childbirth (median)</td>
<td>1996 (1984-2000)</td>
</tr>
<tr>
<td>Miscarriage in past</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>3 (7%)</td>
</tr>
<tr>
<td>No</td>
<td>42 (93%)</td>
</tr>
<tr>
<td>Child with birth defect</td>
<td>4 (9%)</td>
</tr>
<tr>
<td>Fluency of Language:</td>
<td></td>
</tr>
<tr>
<td>English</td>
<td>22 (48%)</td>
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<tr>
<td>Punjabi</td>
<td>23 (51%)</td>
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<tr>
<td>Vegetarianism:</td>
<td></td>
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<tr>
<td>Yes</td>
<td>20 (44%)</td>
</tr>
<tr>
<td>No</td>
<td>25 (56%)</td>
</tr>
</tbody>
</table>

'n' = number of subjects
5.2 FOLATE INTAKE

Seven 24-hour dietary recalls were used to estimate the dietary intake of folate, both natural and synthetic folic acid. Synthetic folic acid intake was calculated by adding the folic acid contributed by fortified foods and found in supplements. The folate intake in this report is expressed as micrograms of synthetic folic acid and/or dietary folate equivalents where applicable. Dietary Folate Equivalent was calculated by multiplying the amount of synthetic folic acid with a factor of 1.67, thus adjusting for the greater bioavailability of synthetic folic acid.

5.2.1 Dietary Intake of Food Folate

The daily mean folate intake was determined by estimating the folate contribution of all food items, fortified or unfortified (Table 2). Adjustments were made for greater bioavailability of synthetic folic acid in fortified foods and intakes are expressed as DFE. Average daily folate intake from food alone was 492 ± 132 DFE/day. More than 90% of the women surveyed were meeting the EAR of 320 DFE/day from dietary sources. The fortification of cereals, bread and grain products increased the average folate intake of the participants from 291 ± 75 DFE/day (Table 2) to 492 ± 132 DFE/day. The number of women meeting the EAR with fortification was 42 (93%) compared to 16 (36%) meeting the EAR without fortification. According to McNemar Test (a test used to determine the difference between pre and post-treatment effects), folic acid fortification significantly increased the proportion of women meeting the EAR (p<0.001). The mean intake of SFA from dietary sources was 121 ± 53 µg SFA/day (range 28 - 266). None of the
participating women met the special recommendation of 400 μg SFA/day from dietary sources alone.

The Recommended Daily Allowance (RDA) for adults is 400 DFE/day from all sources of folate. Thirty-six (80%) women were meeting the RDA from dietary sources with fortification and only three women (7%) were meeting the RDA if the effect of fortification was subtracted. McNemar Test indicates that a significantly higher proportion of women were meeting the RDA as a result of folic acid fortification (p<0.001).

| TABLE 2: Estimated Daily Folate Intakes and Synthetic Folic Acid Intakes Derived From Food Sources With and Without Fortification. (n=45) |
|-------------------------------------------------|---------------------------------|---------------------------------|
| Total folate (DFE/day)                          | Synthetic Folic Acid            |
| With Fortification                              | Without Fortification           |
| Mean ± SD (95% Confidence Interval)            | µg SFA/day                      |
| Mean ± SD                                      | 492 ± 132                      | 291 ± 75                        | 121 ± 53                        |
| Median                                          | (453, 531)                     | (269, 313)                      | (105, 137)                      |
| Range                                           | 482                             | 296                             | 115                             |
| Meeting EAR                                    | 228 - 755                       | 137 - 478                       | 28 - 266                        |
| Meeting Special Recommendation                | 42 (93%)                        | 16 (36%)                        | NA*                             |
| Exceeding UL                                    | 0                               | NA*                             | 0                               |

Note: SD = standard deviation; EAR = Estimated Average Requirement (320 μg DFE/day); Special Recommendation = 400 μg SFA/day; UL = Upper Tolerable Limit (1000 μg SFA/day); * Special recommendation is measured in ‘μg SFA/day’ and not ‘DFE/day’; ** EAR is measured in ‘DFE/day’ and not ‘μg SFA/day’.

According to McNemar Test, folic acid fortification significantly increased the proportion of women meeting the EAR from 36% to 93% (p<0.001).

5.2.2 Synthetic Folic Acid Intake from Supplements

More than half of the participants used some type of nutritional supplement and 19 of them were using a folic acid containing supplement. Of these 19, 18 women indicated that they used the folic acid containing supplement at least once per week.
These women were defined as regular supplement users, and represent approximately 40% of the study participants. The regular use of folic acid containing supplement was not associated with any of the demographic variables on the questionnaire such as age of the women, age of youngest child, language fluency, income, number of years in Canada, etc. The average intake of synthetic folic acid from supplements for regular supplement users was $351 \pm 267 \, \mu g \, SFA/day$ (Table 3). Half of the supplement users met the special recommendation of $400 \, \mu g \, SFA/day$ for women capable of becoming pregnant.

<table>
<thead>
<tr>
<th></th>
<th>Synthetic Folic Acid µg SFA/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (95% Confidence Interval)</td>
<td>351 ± 267 (228, 474)</td>
</tr>
<tr>
<td>Median</td>
<td>343</td>
</tr>
<tr>
<td>Range</td>
<td>71 - 1000</td>
</tr>
<tr>
<td>Meeting Special Recommendation</td>
<td>9 (50.0%)</td>
</tr>
<tr>
<td>Exceeding UL</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; Special Recommendation = 400 \, \mu g \, SFA/day; UL = Upper Tolerable Limit (1000 \, \mu g \, SFA/day)

5.2.3 **Total Folate Intake from Supplements and Food Folate**

Total daily folate intake estimated by combining the dietary folate intake with folate intake from supplements is represented in Table 4. Mean daily folate intake from food folate and folic acid containing supplements was $727 \pm 420 \, DFE/day$. The average SFA intake from all sources combined was $263 \pm 243 \, \mu g \, SFA/day$. A separate analysis was done for supplement users only and the results are presented in Table 4.

When the effect of fortified foods was taken into consideration, 11 women (24% of all participants, including 18 of regular supplement users) met the special recommendation. McNemar Test indicated that folic acid fortification did not
significantly increase the proportion of women meeting the special recommendation of 400 μg SFA/day (p>0.05).

With the addition of the effect of folic acid containing supplements to that of the dietary intake of folate, forty-three women (96%) met the EAR. When a similar analysis was done for regular supplement users only (n=18), all the women met the EAR. According to McNemar test for regular supplement users, use of a folic acid containing supplement did not significantly increase the proportion of women meeting the EAR of 320 DFE/day for adults.

When the effect of folic acid from folic acid containing supplements was added to that of the dietary sources with folic acid fortification, a total of 39 (87%) women were meeting the RDA and all of the 18 regular supplement users met the RDA. Out of the 18 supplement users, 15 (83%) women met the RDA even when the effect of folic acid containing supplement was subtracted. Thus, folic acid containing supplement use did not significantly increase the proportion of women meeting the RDA. In the absence of fortification 17 out of the 18 supplement users met the RDA.

The tolerable upper limit for folate intake is 1000 ug of SFA per day. When only SFA intake from supplements was considered, none of the participants were exceeding the UL (Table 3). Similarly, none of the participants exceeded the UL when SFA intake from fortification only was taken into account (Table 2). When the effect of folic acid fortification was combined with the use of supplements containing supplements two women (4% of all participants and 11% of supplement users only) exceeded the UL (Tables 4 and 5).
TABLE 4: Estimated Daily Folate Intakes and Synthetic Folic Acid intakes From Food Folate and the Use of Folic Acid Containing Supplements for 45 Sikh Women of Childbearing Age Derived from 7-day Dietary Recalls.

<table>
<thead>
<tr>
<th></th>
<th>Total Folate (DFE/day)</th>
<th>Synthetic Folic Acid (µg SFA/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (95% Confidence Interval)</td>
<td>727 ± 420 (604, 850)</td>
<td>263 ± 243 (192, 334)</td>
</tr>
<tr>
<td>Median</td>
<td>601</td>
<td>165</td>
</tr>
<tr>
<td>Range</td>
<td>267 - 2180</td>
<td>41 - 1095</td>
</tr>
<tr>
<td>Meeting EAR</td>
<td>43 (96%)</td>
<td>NA</td>
</tr>
<tr>
<td>Meeting Special Recommendation</td>
<td>NA</td>
<td>11 (24%)</td>
</tr>
<tr>
<td>Exceeding UL</td>
<td>NA</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; EAR = Estimated Average Requirement (320 µg DFE/day); Special Recommendation = 400 µg SFA/day; UL = Upper Tolerable Limit (1000 µg SFA/day)

TABLE 5: Estimated Daily Folate Intake and Synthetic Folic Acid Intake From Food Folate and the Use of Folic Acid Containing Supplements For 18 Sikh Women of Childbearing Age Using a Folic Acid Containing Supplement at Least Once Per Week (n=18).

<table>
<thead>
<tr>
<th></th>
<th>Total Folate DFE/day</th>
<th>Synthetic Folic Acid µg SFA/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD (95% Confidence Interval)</td>
<td>1067 ± 475 (929, 1205)</td>
<td>470 ± 272 (391, 550)</td>
</tr>
<tr>
<td>Median</td>
<td>1076</td>
<td>444</td>
</tr>
<tr>
<td>Range</td>
<td>472 - 2180</td>
<td>114 - 1095</td>
</tr>
<tr>
<td>Meeting EAR</td>
<td>18 (100%)</td>
<td>NA</td>
</tr>
<tr>
<td>Meeting Special Recommendation</td>
<td>NA</td>
<td>11 (61%)</td>
</tr>
<tr>
<td>Exceeding UL</td>
<td>NA</td>
<td>2 (11%)</td>
</tr>
</tbody>
</table>

Note: SD = standard deviation; EAR = Estimated Average Requirement (320 µg DFE/day); Special Recommendation = 400 µg SFA/day; UL = Upper Tolerable Limit (1000 µg SFA/day)

5.2.4 Top Ten Contributors of Folate Intake

The top ten contributors of folate intake in the Sikh women’s diet were, roti, dals, tea, pizza, paratha, white bread, pasta, sag, milk and orange juice, in descending order.

The overall contribution of these ten foods is approximately 70% of all folate consumed by the Sikh women participating in our study. The contribution of each food to total
Folate intake is presented in Table 6. Fortified foods or foods using folic acid fortified flour contributed substantially to daily folate intake. Roti and paratha, which are a type of Indian bread, are made from folic acid fortified wheat flour and together they contributed over 30% of the participant’s daily folate intake. Other foods made from folic acid fortified flour that contributed substantially to the daily folate intake of Sikh women in this study were pizza, white bread and pasta which accounted for almost 9% of all folate consumed by these women. Together these foods accounted for approximately 40% of all folate consumed by these women.

**TABLE 6:** The Folate Contribution of Top Ten Contributors to Folate in the Diet of 45 Sikh Women of Childbearing Age Living in the Lower Mainland, BC, According to 7-Day Dietary Recalls Expressed as Percent Total Folate Intake and Cumulative Percent Total Folate Intake.

<table>
<thead>
<tr>
<th>Ranking</th>
<th>Description of Food item</th>
<th>Folate Contribution (µg)</th>
<th>% Of Total Folate intake</th>
<th>Cumulative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Roti</td>
<td>5265</td>
<td>29</td>
<td>29</td>
</tr>
<tr>
<td>2</td>
<td>Dals</td>
<td>3505</td>
<td>19</td>
<td>48</td>
</tr>
<tr>
<td>3</td>
<td>Tea</td>
<td>1090</td>
<td>6</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>Pizza</td>
<td>726</td>
<td>4</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>Paratha</td>
<td>616</td>
<td>3</td>
<td>61</td>
</tr>
<tr>
<td>6</td>
<td>White bread</td>
<td>560</td>
<td>3</td>
<td>64</td>
</tr>
<tr>
<td>7</td>
<td>Pasta</td>
<td>344</td>
<td>2</td>
<td>66</td>
</tr>
<tr>
<td>8</td>
<td>Sag</td>
<td>335</td>
<td>2</td>
<td>68</td>
</tr>
<tr>
<td>9</td>
<td>Milk</td>
<td>279</td>
<td>2</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>Orange juice</td>
<td>263</td>
<td>1</td>
<td>71</td>
</tr>
</tbody>
</table>
5.2.5 Contribution of Ethnic Foods to Daily Folate Intake

Total contribution of ethnic foods to the daily folate intake of Sikh women's diet is presented in Table 7. Ethnic foods contribute more than 60% of total daily folate intake of the participants and common western foods contribute 37%.

TABLE 7: The Folate Contribution of Ethnic Versus Common Foods in the Diet of 45 Sikh Women of Childbearing Age Living in the Lower Mainland, BC, According to 7-Day Dietary Recall Expressed as Percent Total Folate Intake.

<table>
<thead>
<tr>
<th>Type of Food Item</th>
<th>Total Weight of Food Items (g)</th>
<th>% Total Weight</th>
<th>Total Folate (µg)</th>
<th>% Folate</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; Ethnic Foods</td>
<td>20524</td>
<td>27</td>
<td>11624</td>
<td>63</td>
</tr>
<tr>
<td>&gt; Common Foods</td>
<td>54662</td>
<td>73</td>
<td>6844</td>
<td>37</td>
</tr>
</tbody>
</table>
5.3 Biochemical Determinants of Folate Status

Biochemical determinants of folate status were measured to get a better understanding of the participant’s folate status and are presented in Table 8. RBC folate and plasma homocysteine levels were measured for 41 participants.

5.3.1 Red Blood Cell Folate

The average red blood cell folate level for the participants of this study was 958.2 ± 213.1 nmol/L. All of the participants were within the reference range for red blood cell folate, however, only 23 (56%) women had levels >906 nmol/L, which is associated with reduced risk of NTD.

5.3.2 Plasma Homocysteine

The mean plasma homocysteine level for women in this study was 7.3 ± 2.5 µmol/L and about one third of these women had high plasma homocysteine levels (> 7.8 µmol/L).

<table>
<thead>
<tr>
<th></th>
<th>Red Blood Cell Folate (nmol/L)</th>
<th>Plasma Homocysteine (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>958 ± 213 (893, 1023)</td>
<td>7.3 ± 2.5 (6.5, 8.1)</td>
</tr>
<tr>
<td>95% Confidence Interval</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>942</td>
<td>6.6</td>
</tr>
<tr>
<td>Range</td>
<td>394-1421</td>
<td>4.6-15.8</td>
</tr>
<tr>
<td>Reference Range</td>
<td>125-2500</td>
<td>&lt;7.8</td>
</tr>
</tbody>
</table>

Note: SD = Standard Deviation
5.3.3 Relationship Between Red Blood Cell Folate, Plasma Homocysteine and Dietary Folate Intake

Total folate intake from all dietary sources (natural folate plus synthetic folic acid from fortification) represented as 'DFE/day' significantly correlated with RBC (Red Blood Cell) folate \((r = 0.43; p = 0.005)\), but not with plasma homocysteine \((r = -0.02; p = 0.903)\). A significant positive correlation was also observed between folate intake without fortification and RBC folate \((r = 0.473; p = 0.002)\). Total folate ‘DFE/day’ from diet and supplement combined also correlated significantly with RBC folate \((r = 0.429; p = 0.005)\) but not plasma homocysteine \((r = -0.103; p = 0.521)\). Synthetic folic acid intake from fortified foods and supplements correlated with RBC folate levels when all participants were considered \((r = 0.376; p = 0.016)\), but not when only supplement users were considered \((r = 0.375; p = 0.138)\). There was also a significant negative correlation between the RBC folate (nmol/L) levels and plasma homocysteine (μmol/L) levels \((r = -0.435; p = 0.005)\).

There was a significant linear association between RBC folate (nmol/L) and some measures of folate intake including SFA from diet and supplement, DFE from diet and supplement, and DFE from diet alone (Refer to Figures 6-9; Table 9). A significant negative linear association was also found between RBC folate and plasma homocysteine measures of the participants (Figure 10). No association was seen between folate intake and plasma homocysteine.

If a linear relationship exists, the slope coefficient and constant in each case can be used to write a linear equation for these variables. Thus the value of RBC folate can be predicted by estimating the folate intake.
Figure 6: Linear regression curve between RBC folate and total synthetic folic acid intake from diet and supplement combined, for 41 Sikh women of childbearing age living in the Lower Mainland, BC.

Linear Equation: $\text{RBC Folate} = 871.288 + 0.325(\text{SFA from Diet and Supplement})$

Figure 8: Linear regression curve between the red blood cell folate and total folate intake from diet and supplement expressed as Dietary Folate Equivalents, for 41 Sikh women of childbearing age living in the Lower Mainland, BC.

Linear Equation: $\text{RBC Folate} = 577.099 + 1.324(\text{DFE from diet without fortification})$

Figure 7: Linear regression curve between the red blood cell folate and folate intake from diet expressed as dietary folate equivalents, for 41 Sikh women of childbearing age living in the Lower Mainland, BC.

Linear Equation: $\text{RBC Folate} = 624.905 + 0.679(\text{DFE Diet only})$

Figure 9: Linear regression curve between the red blood cell folate and folate intake from diet without fortification, expressed as dietary folate equivalents, for 41 Sikh women of childbearing age living in the Lower Mainland, BC.

Linear Equation: $\text{RBC Folate} = 803.262 + 0.212(\text{DFE from diet and supplement})$
Figure 10: Linear regression curve between the red blood cell folate and plasma homocysteine, for 41 Sikh women of childbearing age living in the Lower Mainland, BC.

Linear Equation: \( \text{RBC Folate} = 1226.186 + (-36.723)(\text{plasma homocysteine}) \)

TABLE 9: Linear Regression Coefficients Between Red Blood Cell Folate Measure and Folate Intake and Between Red Blood Cell Folate and Plasma Homocysteine Measures of 41 Sikh Women of Childbearing Age Living in the Lower Mainland, BC.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Slope Coefficient</th>
<th>F (39,1)</th>
<th>Significance</th>
<th>Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cell Folate (nmol/L) and SFA from fortified foods + Supplement (SFA/day)</td>
<td>0.325</td>
<td>6.404</td>
<td>0.016</td>
<td>871.288</td>
</tr>
<tr>
<td>Red Blood Cell Folate (nmol/L) and Diet with fortification (DFE/day)</td>
<td>0.679</td>
<td>8.827</td>
<td>0.005</td>
<td>624.905</td>
</tr>
<tr>
<td>Red Blood Cell Folate and Diet + Supplement (DFE/day)</td>
<td>0.212</td>
<td>8.791</td>
<td>0.005</td>
<td>803.262</td>
</tr>
<tr>
<td>Red Blood Cell Folate (nmol/L) and Plasma Homocysteine (µmol/L)</td>
<td>-36.723</td>
<td>9.078</td>
<td>0.005</td>
<td>1226.186</td>
</tr>
<tr>
<td>Red Blood Cell Folate (nmol/L) and Diet without fortification (DFE/day)</td>
<td>1.324</td>
<td>11.270</td>
<td>0.0018</td>
<td>577.099</td>
</tr>
</tbody>
</table>

Note: SFA = Synthetic Folic Acid; DFE = Dietary Folate Equivalents
5.4 Knowledge and Attitudes

5.4.1 Awareness of Folate and the Relationship with Neural Tube Defects

Approximately half of the participants (n=24; 53%) had heard of folate. However, ten of these women were unaware of what folate was and two gave an irrelevant response. Only three women were able to correctly describe folic acid as a vitamin (Table 10). The women that had heard of folate were also asked if they were aware of a relationship between folate and their health. Ten women answered yes, but only two women indicated that folate played a role in the prevention of birth defects. These two women were asked when they thought it was most important for a woman to increase her intake of folic acid in order to reduce the risk of an NTD affected pregnancy. One of them indicated that it was most important to increase the intake of folic acid during childbearing years and the other indicated before pregnancy. Regular supplement users were more likely to have heard of folate compared to the non-users (67% vs 38%; Odds Ratio (OR) = 3.25), as were women that were fluent in English when compared with those that were not (86% vs 22%; OR = 22.8). Non-vegetarian women were more likely to have heard of folate compared to vegetarian women (76% vs 25%; OR = 9.5).


<table>
<thead>
<tr>
<th>Folate is:</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A vitamin</td>
<td>3</td>
</tr>
<tr>
<td>Something needed for pregnancy</td>
<td>3</td>
</tr>
<tr>
<td>Something that prevents birth defects</td>
<td>1</td>
</tr>
<tr>
<td>Something like iron</td>
<td>6</td>
</tr>
<tr>
<td>A nutrient</td>
<td>2</td>
</tr>
<tr>
<td>Don’t know</td>
<td>10</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2</td>
</tr>
</tbody>
</table>
*Note: The total adds up to more than 24 because the women were allowed to give more than one response.

5.4.2 Awareness of Spina Bifida

Eleven women had heard of spina bifida. Most of these women were able to correctly identify spina bifida as being related to problems of the spinal cord/neural tube (n=8), and/or a defect one is born with (n=6) (Table 11). Women fluent in English were more likely to have heard of spina bifida compared with the women that are not fluent in English (45.45% vs 4.35%; OR = 18.3).

TABLE 11: Identification of Spina Bifida by Sikh Women of Childbearing Age Living in the Lower Mainland, BC, Who Reported Having Heard of Spina Bifida (n=11)°.

<table>
<thead>
<tr>
<th>Spina Bifida is:</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A defect that you are born with</td>
<td>6</td>
</tr>
<tr>
<td>Spinal/neural tube problem</td>
<td>8</td>
</tr>
<tr>
<td>Don’t Know</td>
<td>2</td>
</tr>
</tbody>
</table>

°Note: The total adds up to more than 11 because the women were allowed to give more than one response.

5.4.3 Increasing Folate Intake to Reduce Risk of Neural Tube Defects

Most women (n=40; 88.9%) were willing to take a daily supplement of folic acid, if it was demonstrated that folic acid reduces the risk of neural tube defects. On the other hand when asked for the preferred means of increasing folate in their diet, 21 women said a combination of diet and supplement, 18 women chose diet only and six preferred to use supplement only. Current study did not observe any association between the knowledge of folate and current use of supplement.
The women (n = 10), who indicated that they were aware of a relationship between folate and health, were asked if they thought that their diet was providing them enough folate. Two women believed that they got enough folate from diet alone and four did not. Four women were not sure if their diet provided them with enough folate.

All the participants were asked to identify potential barriers to using a daily supplement of folic acid to reduce the risk of NTD. Table 12 lists the various barriers suggested by the participants. ‘Too busy/no desire to change’ was the most commonly reported barrier (n=16; 36%).

<table>
<thead>
<tr>
<th>Barrier</th>
<th>Number (Percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Too Busy/no desire to change current habits</td>
<td>16 (36)</td>
</tr>
<tr>
<td>Financial expense of supplements</td>
<td>4 (9)</td>
</tr>
<tr>
<td>Don’t believe in taking supplements</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Need more information before changing</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Food alone supplies enough folate</td>
<td>2 (4)</td>
</tr>
<tr>
<td>No reason or barriers to taking a supplement</td>
<td>13 (29)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>6 (13)</td>
</tr>
<tr>
<td>Don’t care</td>
<td>4 (9)</td>
</tr>
</tbody>
</table>

Note: Participants were allowed to provide multiple responses

5.4.4 Pregnancy and Use of Folic Acid Containing Supplement

The women who had been pregnant in the past (n=35; 77%) were asked questions about the use of supplements before and during pregnancy. Most of these women did not take any supplements before pregnancy (n=30; 86%), however, the use of supplements during pregnancy was very common (n=32; 91%) and pre-natal supplements were of
choice. Only five women recalled using supplements before pregnancy, one of them used a folic acid supplement and the remaining four used a multivitamin supplement.

The women that did not use supplements before or during pregnancy gave reasons for their choice as listed in Table 13. Three women that did not take supplements during pregnancy said that they felt nauseated and sick after taking them. Fifteen women that did not take supplements before pregnancy indicated that their diet provided them with enough folate at that time and about 12 women indicated that they did not know it was important.

### TABLE 13: Reason Provided for Not Taking a Supplement Before and During Pregnancy Expressed by Sikh Women of Childbearing Age Living in the Lower Mainland, BC, Who Did Not Use a Supplement Before (n=30) or During Pregnancy (n=3).

<table>
<thead>
<tr>
<th>Reason for not taking a supplement</th>
<th>Before pregnancy n = 30</th>
<th>During pregnancy n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Didn’t know that it was that important</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Too busy/ no desire to change current habits</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Doctor didn’t inform of the importance</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Food alone supplies enough nutrients</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>Don’t know</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: Participants were allowed to provide multiple responses

### 5.4.5 Sources of Information on Folate

The women that had heard of folate were asked to recall where they learned about it. The various sources of information are listed in Table 14. Most women had reported having heard of folate from their doctor (n=11; 46%).
TABLE 14: Common Sources of Information on Folate Reported by Sikh Women of Childbearing age Living in the Lower Mainland, BC, who Had Heard of Folate (n=24).

<table>
<thead>
<tr>
<th>Information source</th>
<th>Number (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doctor</td>
<td>11 (46)</td>
</tr>
<tr>
<td>School</td>
<td>6 (25)</td>
</tr>
<tr>
<td>Magazines/newspapers</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Family/spouse</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Friends</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Television/radio</td>
<td>0</td>
</tr>
<tr>
<td>Other health care professionals</td>
<td>0</td>
</tr>
<tr>
<td>Pamphlets</td>
<td>0</td>
</tr>
<tr>
<td>Internet</td>
<td>0</td>
</tr>
<tr>
<td>Other Sources</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: Participants were allowed to provide multiple responses

Table 15 lists preferred sources of information on folic acid and spina bifida as identified by the participants. All participants identified family doctor as a preferred source of information. A majority of them also indicated that television/radio (n=30; 67%) and magazines/newspapers (n=29; 64%) would be desirable sources of information.

TABLE 15: Preferred Sources of Future Information on Folate Reported by All Sikh Women of Childbearing Age Living in the Lower Mainland, BC (n=45).

<table>
<thead>
<tr>
<th>Information source</th>
<th>Number (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family Doctor</td>
<td>45 (100)</td>
</tr>
<tr>
<td>Television/radio</td>
<td>30 (67)</td>
</tr>
<tr>
<td>Magazines/newspapers</td>
<td>29 (64)</td>
</tr>
<tr>
<td>Friends</td>
<td>25 (56)</td>
</tr>
<tr>
<td>Food labels</td>
<td>23 (51)</td>
</tr>
<tr>
<td>Pamphlets</td>
<td>21 (47)</td>
</tr>
<tr>
<td>Media campaign</td>
<td>20 (44)</td>
</tr>
<tr>
<td>Pharmacist</td>
<td>19 (42)</td>
</tr>
<tr>
<td>Family/spouse</td>
<td>16 (36)</td>
</tr>
<tr>
<td>Nutritionist</td>
<td>16 (36)</td>
</tr>
<tr>
<td>Other</td>
<td>15 (33)</td>
</tr>
<tr>
<td>Package inserts</td>
<td>12 (27)</td>
</tr>
<tr>
<td>Health food store</td>
<td>9 (20)</td>
</tr>
</tbody>
</table>

Note: Participants were allowed to provide multiple responses
Only nine women recalled their family doctor talking to them about folate. Eight of these women claimed that their doctor suggested they use supplements in order to increase their folate intake. Only one woman recalled her doctor suggesting the use of diet and supplements to increase folate intake. Six women recalled their doctor recommending them to increase their folate intake during pregnancy. Three women reported that their doctor asked them to increase their folate intake before pregnancy. However, none of the doctors recommended that folate intake be increased during the childbearing years.

5.4.6 Identification of Folate Food Sources

All women that reported having heard of folate (n = 24) were asked to identify what they believed to be good sources of folate from a list of nine food items. A score of +1, 0, or −1 was assigned to each answer and the scores on all nine food items were then summed up to get the final total score for the individual. This score was then used as an indicator of the participant's knowledge of food sources of folate. The women's score on this question ranged from −3 to +7. Based on the scores, women were grouped into one of four categories, 'very poor' (score <0), 'poor' (score 0-3), 'good' (score 4-7), or 'very good' (score 7-9). According to Table 16, half of the women scored 'good' (n=13; 54%) and almost the same number of women scored poor (n=10; 42%).

Foods most often correctly identified as good sources of folate were broccoli, breakfast cereals, oranges, asparagus and dried beans and peas. Beef was most often identified as 'don't know' (Table 17).
TABLE 16: Knowledge of Foods that are Good Sources of Folate Reported by 24 Sikh Women of Childbearing Age Living in the Lower Mainland, BC, Who Had Heard of Folate (Maximum Score of Nine Points Possible).

<table>
<thead>
<tr>
<th>Knowledge of foods containing folate (score out of 9)</th>
<th>Number (percent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor (&lt;0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>Poor (0-3)</td>
<td>10 (42)</td>
</tr>
<tr>
<td>Good (4-6)</td>
<td>11 (46)</td>
</tr>
<tr>
<td>Very good (7-9)</td>
<td>2 (8)</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Food item</th>
<th>Yes n</th>
<th>No n</th>
<th>Don’t know n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oranges</td>
<td>15</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Beef</td>
<td>3</td>
<td>5</td>
<td>16</td>
</tr>
<tr>
<td>Chocolate</td>
<td>1</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Asparagus</td>
<td>15</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Broccoli</td>
<td>19</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Grapes</td>
<td>9</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>16</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Garlic</td>
<td>3</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Dried beans and Peas</td>
<td>14</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

5.4.7 Awareness of Folic Acid Fortified Foods

Table 18 presents the participants awareness of foods fortified with folic acid. Most women scored ‘poor’ or ‘very poor’ (n=16; 67%). Breakfast cereals were most often identified as being fortified with folic acid (n=39; 87%) (Table 19). Apple juice (n=31; 69%) and milk (n=31; 69%) were most often wrongly identified as being fortified with folic acid.
TABLE 18: Knowledge of Foods that are Fortified With Folic Acid Reported by 24 Sikh Women of Childbearing Age Living in the Lower Mainland, BC, Who Reported Having Heard of Folate (Maximum Score of Seven Points Possible).

<table>
<thead>
<tr>
<th>Knowledge of foods fortified with Folic acid (Score out of 7)</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor (&lt;0)</td>
<td>8</td>
</tr>
<tr>
<td>Poor (0-2)</td>
<td>8</td>
</tr>
<tr>
<td>Good (3-4)</td>
<td>6</td>
</tr>
<tr>
<td>Very good (5-7)</td>
<td>2</td>
</tr>
</tbody>
</table>


<table>
<thead>
<tr>
<th>Food item</th>
<th>Yes n</th>
<th>No n</th>
<th>Don’t Know n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>23</td>
<td>21</td>
<td>1</td>
</tr>
<tr>
<td>Apple juice</td>
<td>31</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Rice</td>
<td>11</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>Pasta</td>
<td>24</td>
<td>20</td>
<td>1</td>
</tr>
<tr>
<td>Milk</td>
<td>31</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>Margarine</td>
<td>18</td>
<td>24</td>
<td>3</td>
</tr>
<tr>
<td>Breakfast cereals</td>
<td>39</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

5.4.8 Sikh Women’s Attitudes and Perceptions About Diet and Pregnancy

Forty-one of the participants believed that diet plays a role in pregnancy and 34 would attempt to change their diet during pregnancy. The participants identified a number of food items that they would add to their usual diet. Some of the more popular foods that would be added to the diet and those that would be avoided from the diet during pregnancy are identified in Table 20.

The participants were also asked about their beliefs around the concept of “hot” and “cold” foods. More than half of the women surveyed believed in this concept (n = 24;
53%). Table 21 lists the food items most commonly identified as hot or cold by the study participants.

TABLE 20: Foods Most Commonly Added to the Regular Diet or Avoided From the Diet During Pregnancy by Sikh Women of Childbearing Age Living in the Lower Mainland, BC (n=45).

<table>
<thead>
<tr>
<th>Foods Added</th>
<th>Foods Avoided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milk</td>
<td>Sweets/ Sugars</td>
</tr>
<tr>
<td>Fruits</td>
<td>Fried Foods</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Spices</td>
</tr>
<tr>
<td>Juices</td>
<td>High Fat Foods</td>
</tr>
<tr>
<td>Yogurt</td>
<td></td>
</tr>
<tr>
<td>Chicken</td>
<td></td>
</tr>
</tbody>
</table>

TABLE 21: Foods Most Commonly Identified as ‘Hot’ or ‘Cold’ by Sikh Women of Childbearing Age Living in the Lower Mainland, BC (n=45).

<table>
<thead>
<tr>
<th>Hot Foods</th>
<th>Cold Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic</td>
<td>Yogurt</td>
</tr>
<tr>
<td>Ginger</td>
<td>Buttermilk</td>
</tr>
<tr>
<td>Ginger powder</td>
<td>Milk</td>
</tr>
<tr>
<td>Mangoes</td>
<td>Oranges</td>
</tr>
<tr>
<td>Almonds</td>
<td>Potatoes</td>
</tr>
<tr>
<td>Raisins</td>
<td>Melons</td>
</tr>
<tr>
<td>Spices</td>
<td>Juices</td>
</tr>
<tr>
<td>Bitter melon</td>
<td>Black gram</td>
</tr>
<tr>
<td>Muth</td>
<td>Rice</td>
</tr>
<tr>
<td></td>
<td>Squash</td>
</tr>
<tr>
<td></td>
<td>Watermelon</td>
</tr>
</tbody>
</table>
CHAPTER 6: DISCUSSION

6.1 Overview of Results

A sample of 45 Sikh women of childbearing age (18-45 years) living in the Lower Mainland of BC was studied to estimate their folate status, knowledge, and attitudes and perceptions about diet and pregnancy. This is the first study of its kind to examine the folate status of Sikh women in the Lower Mainland, BC. In fact few studies have examined these parameters in Sikh women around the world, even though they have a high prevalence of giving birth to NTD affected infants. The highlights of findings from this study include:

• Fortification increased intake by about 100 µg as predicted prior to the mandatory fortification of flour with folic acid.

• When total folate intake was considered, 96% of this population met the EAR (Estimated Average Requirement of 320 DFE/day).

• When total synthetic folic acid intake was calculated, 24% women met the special recommendation of 400 µg SFA/day to reduce the risk of NTD affected pregnancy.

• Only about half of the women had red blood cell folate levels > 906 nmol/L (levels associated with reduced risk of NTD) and about one third had high plasma homocysteine levels (> 7.8 µmol/L).

• Twenty-four women had heard of folate but only two were aware of the relationship between folate and spina bifida.

• Most common sources of folate information were doctors, magazines/newspaper, and school.
• Half of the participants did not take a supplement because many were not aware that they should take one or that they believed their diet supplied enough folic acid.

• Regarding pregnancy, 41 women believed that diet played an important role, 34 reported that they would change their diet during pregnancy and more than half believed in the concept of hot and cold foods.
6.2 Compliance and Demographic Characteristics

Out of the 50 women who were originally recruited in this study, 45 (90%) completed the dietary assessment and knowledge and attitudes questionnaire. The 90% compliance rate of our study is comparable to other studies assessing dietary intake of folate and/or knowledge of folate among women of childbearing age using non-random/convenience sampling techniques (Draper et al., 1993; Firth et al. 1998; French et al., 2003). Draper et al., (1993) assessed energy and nutrient intakes of different types of vegetarians with a compliance rate of 93%. Firth et al., (1998) used a convenience sample of 25 women of childbearing age (18-45 years) to estimate individual folate intakes and observed 84% compliance rate as 21 women completed the study. The slightly lower compliance rate of Firth’s study may be explained by higher respondent burden in their study and the use of a different method of dietary data collection when compared to the present study. Firth et al., collected 14 food records over a period of 60 days as opposed to 7 day dietary recalls collected over four weeks as done in this study. In their validation study French et al., (2001) used 7-day dietary records over a 4-week period and observed a compliance rate of 85%, which is similar to that of Firth’s study and lower than that of the present study.
6.3 Folate Intake and NTD Risk

6.3.1 Dietary Folate Intake

To date there are limited data available on folate and/or folic acid intake of Sikh women worldwide. One study conducted in the UK looked at the nutrient intake of Moslem, Sikh and Hindu pregnant women in Birmingham (Wharton et al., 1984). The authors estimated an average intake of folate to be 130.7 (57-389) μg folate/day for Sikh women (n=15). In another study conducted in India (Panwar and Punia, 1998) the average folate intake of 90 pregnant women of Haryana (neighboring state of Punjab) was approximately 200 μg folate/day. If we subtract the effect of folic fortification from our study data, the average folate intake would be 291 ± 75 DFE/day (Which may be equated to 291 ± 75 μg folate/day), which is still higher than the above two studies. Thus, results of previous studies differ from the dietary intake of folate observed in the current study for the following reasons. The difference in the results could be due to differences in geographical location of the studied population and that the women were pregnant in the previous studies as opposed to non-pregnant women in the present study. This may be an important factor because the diet of pregnant women is generally different from that of non-pregnant women.

In the current study, 93% (n=42) of the participants were meeting the EAR of 320 DFE/day and 80% (n=36) met the RDA of 400 DFE/day. Boushey et al., (2001) conducted a cross-sectional study using a quantitative food frequency questionnaire to estimate the dietary intake of 284 women between the ages of 18-89 years in the US. They reported that 94% of women between the ages of 18-46 years were meeting the
EAR of 320 μg DFE/day from dietary sources alone. In another study conducted by Lewis et al., (1999), 85% women between the ages of 20-49 years were meeting the EAR. Boushey et al., have demonstrated that younger women (between the age groups of 18-39 years and 40-46 years) tended to eat more servings of bread and cereals compared to women from the older age group (55-89 years). Thus, the proportion of women that met the EAR in all of the above studies appears to similar to that observed by the current study.

French et al., (2003) estimated dietary folate intake of 148 women of childbearing age living in lower mainland, BC. They observed that 77% of the participants were meeting the EAR for folate, which appears lower than our estimate of 93%. The difference in sampling procedure, the sample size itself, data collection methods, and consumption of cereals and legumes differing due to ethnic backgrounds, may be responsible for this discrepancy in the results of these two studies that were conducted in the same geographic location and among women of similar age.

French et al., (2003) used a random sample consisting of a predominantly white population who had higher incomes and higher education than the present study. The present study, on the other hand, used a convenience sample of all Sikh women with fairly even distribution of income and education levels. French et al., also used a different method of data collection compared to the present study. They used a semi-quantitative food frequency questionnaire as opposed to seven-day dietary recalls used in this study. Also our population had a dietary pattern very different from the population involved in French’s study. Women in our study consumed a predominantly ethnic/traditional Punjabi diet, made up of “roti/paratha” (made for wheat flour) and “dals” (i.e. legumes
and lentils) which are generally recognized as important sources of folate. Over all ethnic foods contributed significantly to daily folate intake of Sikh women of our study and were responsible for 63% of total daily folate intake. However, the mean folate intake from dietary sources estimated by French et al., (2003), (470 ± 200 DFE/day) was comparable to the present findings.

6.3.2 Major Food Contributors of Folate

In this study, the top ten foods contributing to the folate intake of Sikh women of childbearing age were identified to be roti, dals, tea, pizza, paratha, white bread, pasta, sag, milk and orange juice. French et al., (2003) also reported tea, pizza, white bread, pasta, milk and orange juice as top contributors to the folate intake of women living in the lower mainland of BC, however, this study revealed that more ethnic foods such as dal, roti, paratha and sag were also important sources of folate for this population. Fortified foods identified as major contributors included roti, pizza, paratha, white bread and pasta. Together these items represented about 41% of the participant's daily folate intake, suggesting that folic acid fortified foods make a substantial contribution to the diet of Sikh women. French et al., (2003) also reported rice in their top contributors. Even though rice is a major food in the diets of Sikh women, the type of rice that they prepare and consume is Basmati rice, which is not precooked and thus, not fortified. On the other hand, French et al., (2003) presumed that all rice used by their participants was fortified with folic acid. In French’s study, fortified foods accounted for 26% of the daily folate intake.
6.3.3 Role of Fortification in Increasing the Dietary Intake of Folic Acid

In the present study folic acid fortification increased the average daily folate intake from an estimated 291 ± 75 DFE/day to 492 ± 132 DFE/day, thus increasing the number of women meeting the EAR from 36 % (n=16) to 93% (n=42). Prior to the mandatory fortification of folic acid, it was hypothesized that fortification of bread and grain products would result in an increase of approximately 100 µg of folic acid per day in the diet of an adult woman (Gregory, 1997). In the current study 40% of the total folate intake was attributable to folic acid fortified foods and fortification resulted in a mean increase of folic acid intake by 121 ± 53 µg SFA/day, approximately what was initially anticipated and similar to what others have found (French et al., 2003; Boushey et al., 2001). Boushey et al., (2001) estimated an increase in intake of 180 to 187 µg SFA/day for women between the ages of 18 and 45 years, an amount that is higher than the present findings (121 ± 53 µg SFA/day). The difference may be explained by differences in research methods of these studies. Boushey and colleagues studied 284 women from the rural Midwest region of US recruited through health screening programs and used a quantitative food frequency questionnaire to collect dietary intake data. They modified the data to reflect folic acid fortification by using the formula ([D X 140 µg folic acid]/100g flour) where D is the estimated number of grams per day of flour.

6.3.4 Concerns of Exceeding the Tolerable Upper Intake Level Due to Fortification

One of the concerns with the folic acid fortification program was increasing a proportion of the population with intakes that exceed the upper intake level for folic acid (1000 µg SFA/day) The tolerable upper intake level for folic acid is set at a level that is
intended to minimize the risks associated with excessive folate intake. This high folate contribution from fortified foods and the fact that the prevalence of vegetarianism among Sikh women is high, make it important to monitor vitamin B₁₂ in this population due to the potential for B₁₂ deficiency and the possible masking of this condition. Too much folic acid could prevent a hematologic response in people who have a vitamin B₁₂ deficiency. This could result in the masking of megaloblastic anemia associated with vitamin B₁₂ deficiency and consequently could delay the appropriate treatment. Because the neuropsychiatric complications of B₁₂ deficiency do not respond to folate, they can progress in the absence of B₁₂ therapy (Rothenberg, 1999). Another potential concern is that folate may interact with antiepileptic therapy. In the present study none of the women exceeded the UL of 1000 μg SFA/day through the consumption of fortified foods alone. French et al., (2003) and Boushey et al., (2001) also reported that none of their study participants exceeded the UL with the use of fortified foods alone. The highest amount of synthetic folic acid consumed from fortified foods in our study was 266 μg SFA/day, which is far from the UL of 1000 µg SFA/day. In 1999, Kloeblen reported that 20% of low-income, predominantly minority pregnant women had a daily folate intake greater than 1000 μg folic acid, solely from fortified grain products. However, this population consisted of pregnant women who tend to have higher nutrient intakes per day than non-pregnant women. As well, this study was conducted in the US where ready-to-eat cereals can be fortified at a higher level than those in Canada. And lastly, these authors reported the intake as total folate (μg folate/day), whereas the current UL is 1000 μg SFA/day. In our study even when the effect of supplementation was included in the estimation of synthetic folic acid intake, only two women (4%) exceeded the UL. These
results compare favorably with those of French et al., (2003) and Lewis et al., (1999), who found 7% and 5%, respectively, exceeding the UL.

6.3.5 Increasing the Level of Folic Acid Fortification to Further Reduce NTD Risk

At the current level of fortification the mean synthetic folic acid intake for our population of Sikh women of childbearing age was about 120 μg SFA/day (range: 28 - 266 μg SFA/day) from fortification alone. Thus, even the woman with the highest level of intake in our study was unable to meet the special recommendation of 400 μg SFA/day by using folic acid fortified foods alone. Similar results have been demonstrated by other studies (French et al., 2003; Boushey et al., 2001; Lewis et al., 1999). Although the current level of fortification is not sufficient to help women of childbearing age meet the special recommendation on its own, Daly et al., (1997) demonstrated that an increase in folic acid intake by 100 μg SFA/day (equivalent to the effect of folic acid fortification) would reduce the NTD risk per 1000 births by 22%, and this has been demonstrated by Honein et al., (2001), who reported that 19% reduction in the NTD birth prevalence following fortification in the US. In Canada, Gucciardi and colleagues have reported an even higher reduction (50% reduction; from 8.6 per 10000 live births in 1986 to 4.3 per 10000 live births in 1999) in the NTD birth prevalence. Also, as fortification provides a constant inevitable exposure, the cumulative effect of lower doses could be greater and over time could result in red blood cell folate values associated with even lower risk of NTD.

Daly et al., also predicted that doubling the intake of synthetic folic acid to 200 μg SFA/day could further reduce the NTD risk to 41%. Thus, doubling the fortification level
could potentially result in further reducing the risk of NTD. We have demonstrated that red blood cell folate levels are inversely related to plasma homocysteine levels in our study and thus we may see a reduction in the levels of plasma homocysteine levels also concurrent with the increase in red blood cell folate levels. Thus an increase in fortification levels may potentially benefit by reducing the risk of NTD affected pregnancies and also reducing the risk of developing cardiovascular disease (Misra et al., 2002; Bailey et al., 2003).

Increasing the level of fortification may increase the risk of exceeding UL and as a result mask B_{12} deficiency in some groups. If we were to double the level of fortification as discussed above, we would still not have any woman exceeding the UL by consumption of fortified foods alone. And if we added the effect of supplement, we would still have only two women exceeding the UL. However, caution must be exercised to monitor the elderly population due to the higher risk of masking B_{12} deficiency in this population. It has also been suggested that B_{12} be added to the foods fortified with folic acid in order to reduce the chances of masking B_{12} deficiency. (Oakley, 1997; Ray et al., 2000)

Thus, we may argue that the current level of fortification is sufficient, especially if the intake of SFA from fortified foods is complemented with the use of a daily folic acid containing supplement.

6.3.6 Supplementation with Folic Acid:

Results from this study and others show that folic acid fortification did not increase the proportion of women of childbearing age meeting the special
recommendation of 400 µg SFA/day. Thus, it may be necessary for women of childbearing age to take a daily supplement to guarantee synthetic folic acid intake at a level that will meet the special recommendation for NTD risk reduction. However, it has been demonstrated that the current level of fortification has resulted in increasing the red blood cell folate levels of women of childbearing age to >906 nmol/L (Ray et al., 2002; Kapur and Koren, 2000; Caudill et al., 2001) and reduction of NTD risk (Honein et al., 2001; Ray et al., 2002; Gucciardi et al., 2002). Thus, this level of fortification may be sufficient, especially if combined with supplement use.

For instance, when intake from supplementation was added to that of fortification, 11 women (24% of all participants) in the present study met the special recommendation. Other studies have shown similarly that one quarter to one third of the general population of women of childbearing age met this recommendation when supplement intake was added into the equation. (French et al., 2003; Firth et al., 1998)

Even though supplementation is an important route for ensuring that women of childbearing age meet this special recommendation, a large proportion of women are not using folic acid containing supplements on a regular basis. In the present study 40% (n=18) of Sikh women of childbearing age were using folic acid containing supplements on a regular basis, i.e. at least once a week, and only half (n=9, 20% of all participants) of these regular supplement users consumed the supplement daily. However, French et al., (2003) showed that only 28% of their participants in the Lower Mainland, BC were using a daily supplement. In earlier studies in the US, about one third of women were reported to be supplement users (Firth et al., 1998 and Center for Disease Control and Prevention, 1999). In a more recent study, Boushey et al., (2001) reported that 54% of women were
consuming folic acid containing supplements. This increase in usage could be attributed to the effect of public education campaigns and media campaigns in the US.

A number of potential barriers against regular supplement use among Sikh women of childbearing age were identified in this study. These women identified being too busy or no desire to change, lack of awareness and not believing in taking supplements as potential barriers. Other barriers identified less frequently were financial expense, carelessness and belief that food alone supplies enough folate. These barriers are consistent with those identified by a predominantly Caucasian group of women as presented in the study by French and colleagues (2003), however, they did report financial expense as one of the major barriers. Other studies conducted in the US (Centers for Disease Control and Prevention, 1999) and Europe (McGovern et al., 1997; de Jong-Van den Berg et al., 1998) have also identified similar barriers.

The March of Dimes conducted a study with American women of childbearing age in 1997, and reported that more than half of their participants (56%) that did not use supplements either had “no particular reason” or did not feel the need for it. About the same proportion reported that they needed more information (58%) or that cost was a potential barrier (60%). In a 1997 Australian study of women of childbearing age, the preference to get folate naturally from food was identified as the most popular (59%) reason for not taking folic acid supplements and a dislike for taking supplements (12%) was the next popular reason (Bower et al., 1997).

Thus, barriers faced by Sikh women in this study are not unique to them but typical of women of childbearing age all over the world. As a result, we need to address these issues in order to encourage more women to take supplements containing folic acid.
on a regular basis to reduce the risk of NTD affected pregnancies. A behavioral change of this nature to improve the proportion of women using supplements containing folic acid on a regular basis is a complex and lengthy process and it may be possible that increasing the women’s awareness may facilitate this process of behavioral change.

6.3.7 Red Blood Cell Folate Levels

The average red blood cell folate level for the study population in the current study was 958 ± 213 nmol/L. There are no studies that have documented the red blood cell folate levels of Sikh women in North America. However, in the UK, Michie et al., (1998) estimated the red blood cell folate level of women of Pakistani and Indian origin to be 246 ± 87 ng/mL (558± 198 nmol/L). It is important to note that Michie et al.’s study was done in the UK where there was no mandatory folic acid fortification at the time. Their findings seem to be comparable to the pre-fortification levels of red blood cell folate of normocytic women between the ages of 15 to 45 years living in Ontario. Kapur and Koren (2001) reported that the mean red blood cell folate level for these women was 517 ± 215 nmol/L. They also observed that these levels increased by almost two-fold following fortification to 901 ± 318 nmol/L. This value is more in line with the red blood cell folate levels reported in our study. However, it must be noted here that the authors reviewed the red blood cell folate levels from samples that were already collected. Besides, the authors did not comment on the reasons for the collection of these samples from the women. Unlike Kapur and Koren’s study, we lack data on pre-fortification red blood cell folate levels of our participants, thus we are unable to comment on the effect of folic acid fortification on red blood cell folate levels of these women. Unlike Kapur and
Koren, Ray et al., (2002) did not observe as high increases in red blood cell folate due to fortification in women of childbearing age living in Ontario. They reported that red blood cell folate levels increased from a mean of 527 nmol/L prior to fortification to 741 nmol/L after fortification. Ray et al., reviewed the blood records from community-based laboratory and Kapur and Koren had used a clinical laboratory record. Thus, the population group that they were reviewing was potentially different from one another. Also, Ray et al., actually had begun their post fortification blood collection before fortification became mandatory in Canada, which may have resulted in the lower post fortification observations in their study. A US study conducted by the Center for Disease Control and Prevention (1999) reported results similar (715 nmol/L) to those of Ray et al.’s. But, the pre-fortification red blood cell folate levels (411 nmol/L) were lower than that reported by Ray et al., and also by Kapur and Koren. In another US study, Caudill et al., (2001) investigated the folate status of women of childbearing age in Southern California after folic acid fortification and reported an average red blood cell folate level of 1307 ± 349 nmol/L, which is higher than results found in this study and others. More than 40 % of the participants in the present study had red blood cell folate levels below 906 nmol/L, a level associated with very low NTD risk (Daly et al., 1995), whereas 22% of the women in Caudill’s study were below this level. The difference in results of these studies could be explained partially by the different assays used to measure red blood cell folate (Sauberlich, 1999). Caudill et al., used a microbial assay for the red blood cell folate assessment and all other studies mentioned above including the present study had used a radioassay.
6.3.8 Plasma Homocysteine Levels

Another biochemical parameter associated with increased NTD risk and folate metabolism is plasma homocysteine. Hyperhomocysteinemia is associated with NTD risk. The average plasma homocysteine level in our study was $7.3 \pm 2.5 \mu\text{mol/L}$, higher than that of Caudill et al.'s (2001) mean plasma homocysteine level of $5.5 \pm 1.7 \mu\text{mol/L}$. Our result is comparable to the mean homocysteine level of white women in Gerhard et al.'s study (1999), but lower than the mean homocysteine levels of black women in their study ($8.32\mu\text{mol/L}$). It is also lower than the post fortification homocysteine levels observed by Jacques et al., (1999) of $9.4 \mu\text{mol/L}$.

There is evidence indicating that there may be genetic variations in homocysteine levels (Chambers et al., 2000; Gerhard et al., 1999) and specifically there is indication for increased prevalence of hyperhomocysteinemia among men of Northern Indian decent (Chambers et al., 2000). Chambers et al., (2000) conducted two parallel case-control studies [men with Coronary Heart Disease (CHD) as cases and healthy men as controls], one with European men (294 cases and 507 control) and another with Indian Asian men (257 cases and 518 control) in London, UK. Fasting and post-methionine load homocysteine, vitamin B$_{12}$ and folate concentrations were measured as well as conventional CHD risk factors. They observed that fasting homocysteine concentrations were $0.6 \mu\text{mol/L}$ higher in Indian Asians than European controls. The main determinant of this difference appeared to be vitamin B$_{12}$ concentrations. There is no similar study that has examined plasma homocysteine status of South Asian Indian women of childbearing age.
6.3.9 Increased Folic Acid Intake and Incidence of NTD

Although our results indicate that fortification increased synthetic folic acid intake among Sikh women of childbearing age at levels, this increase did not help more women meet the special recommendation of 400 μg SFA/day for NTD risk reduction. Similar results have been reported by French et al., (2003) and Boushey et al. (2001). The minimal effect of folic acid fortification on the proportion of women of childbearing age meeting the special recommendation for NTD risk reduction must not be automatically presumed as ineffectiveness of folic acid fortification in reducing the NTD risk. There are studies from the US that have demonstrated a reduction in the rate of NTD since fortification. Honein et al., (2001) reported a 19.6 % decrease in NTD prevalence following fortification. The authors do warrant that factors other than fortification may have a role to play as well. Van Allen has suggested better screening technology and thus, early terminations of pregnancy and prevalence is reported as per live births. As well, the red blood cell folate levels have increased from what has been reported in the past, etc.

The uneven reporting practices make it difficult to determine trends in the incidence of NTD's. Currently there are three separate reporting agencies. The Canadian Pediatric Surveillance Program, the Canadian Congenital Anomalies Surveillance System, and the Ontario Maternal Serum Screening Program. Also due to the voluntary nature of reporting, it is documented that NTD's are under reported in Canada (Elnarson et al., 2000). Recently, Gucciardi et al., (2002) reported that the total NTD incidence rate had increased from 11.7 per 10,000 pregnancies in 1986 to 16.2 per 10,000 pregnancies in 1995 (pre-fortification), and then decreased to 8.6 per 10,000 pregnancies in 1999.
(post fortification). The increase in the NTD incidence rate from 1986 to 1995 may be explained by increased prenatal screening and better detection of NTDs. The present decrease in the incidence may be due to the folic acid fortification program by Health Canada.
6.4 Knowledge and Behaviour

6.4.1 Awareness of Folic Acid and Neural Tube Defects

Fifty three percent (n = 24) of the Sikh women participating in the present study had heard of folate and three of them identified it as a vitamin. Three women said it was related to pregnancy and only one said that it prevents birth defects. The level of awareness of women in this study is comparable to the results of a South American study conducted by Castilla and de Graca Dutra, 1997. Only 14% of 2806 South American women surveyed knew of the beneficial effects of folate and less than 1% were aware that folate is beneficial to the fetus. Kloebelen et al., (1999) surveyed low income pregnant women in the US and found that 51% women had heard of folate which is similar to the level of awareness among Sikh women in our study, however, in their study 73% women that had heard of folate were aware of the relationship between folate and birth defects and 46% were able to correctly describe folate, which is much higher than in our study. The fact that these women were already pregnant may have contributed to a higher level of knowledge about folate in this group.

To date there is no other study conducted that assessed Sikh women’s awareness of folate and its relationship to NTD. In a recent study of married, well educated women belonging to an upper class in Delhi only 10 out of the 50 women surveyed had heard of folate (Gupta and Gupta, 2000). The level of awareness in this group was much lower compared to that of Sikh women of childbearing age in our study.

French et al., (2003) reported that 95% of the study participants had heard of folate or folic acid and more than half of the women surveyed had some awareness of
what folic acid was. Twenty percent of the respondents knew that folate was a vitamin and 36% reported that folate is something needed for pregnancy. This level of awareness is higher than a number of other studies in North America (Centers for Disease Control and Prevention, 1999; McDonnell et al., 1999). In another study conducted in the Lower Mainland, BC, Morin et al., (2001) reported that 47% of the women they surveyed identified folic acid as one vitamin specifically associated with reduction of birth defects. Their results are much higher compared to that our study and also French et al.’s. The fact that they used a multiple-choice questionnaire for their survey against an open-ended questionnaire used by us may be responsible for the higher levels of awareness reported by them (and their women had been pregnant recently). Morin et al., (2001) also reported that 20% East Indian women surveyed by them knew that vitamins could prevent birth defects. They also reported that women from ethnicities other than Caucasian were less informed about the usefulness of folic acid. Dawson et al., (2001) conducted a survey among women attending a genetics clinic at an Ottawa pediatric hospital and found that 81% of the women in their study were aware of folic acid. Seventy-eight per cent of women in their study were using folic acid containing supplements, but only 26% pregnant women began supplementation early enough to reduce the risk of neural tube defects. Awareness about the vitamin may be higher since these women were attending a genetics clinic.

In the current study, regular supplement users were more likely to have heard of folate than non users however, there was no difference in the proportion of women that had heard of NTD or spina bifida among regular supplement users and non-users. A higher proportion of women that were fluent in English and those who were non-
vegetarians had heard of folate and NTD. As well, more women with children, younger women and unmarried women had heard of NTD, but not folate. There was no association between knowledge of folate/NTD and supplement use in our study.

Only two women reported that folate plays a role in prevention of birth defects and one of them correctly identified that folic acid intake should be increased during childbearing years and the other woman reported before pregnancy. Since there were only two women, there was not the potential to compare the results with other studies and to detect the influence of various demographic variables.

6.4.1.1 Sources of Information

Sikh women that had heard of folate in the current study were asked to recall their source(s) of information. Common sources of information on folate included doctor, school, magazines and friends and families. At the time of data collection, informational pamphlets were being used in British Columbia to disseminate information on folate to women of childbearing age; however, none of our women identified them as their source of information on folic acid.

The sources of information identified by the Sikh women in this study are similar to those identified by women in other studies conducted in the Lower Mainland, BC (French et al., 2003; Morin et al., 2001), and other parts of Canada (Dawson et al., 2001; Neimanis et al., 1999). Studies with women in other parts of the world (Chan et al., 2001; Bower et al., 1997; Wild et al., 1996; McGovern et al., 1997; Sayers et al., 1997) show similar findings as well.
Although there are no studies that have assessed the knowledge of Sikh women in BC or around the world, in a study done by Gupta and Gupta (2000) in New Delhi, India, all the women that had heard of folate reported that they had heard of it from their doctor. Thus, it appears that Sikh/Indian women tend to rely on their doctors as other women do for information on folate.

All women in our study were asked to indicate the preferred sources of future information on folic acid and spina bifida for Sikh women. All women reported the family doctor as the preferred source of information for Sikh women. Other sources identified in the order of their popularity were television/radio, magazines/newspapers, friends, food labels, pamphlets, media campaign, pharmacist, nutritionist, family/spouse and package inserts. Most women also indicated here that the information must be translated into Punjabi so that the information can be accessed by most Sikh women of BC. Since most women recalled having heard of folate from their family doctor and all women indicated them as the preferred source of information, targeting family physicians to educate Sikh women on the importance of folic acid could be successful.

6.4.1.2 Role of the Physician in Folic Acid Education

Physicians were the most preferred source of information among Sikh women of BC, as all 45 participants reported that they would prefer to learn about folic acid from their doctors in the future. However, only nine women in the current study reported having discussed folic acid with their doctor. Eight of these women recalled their doctor recommending them to increase folic acid intake through supplements and one of them recalled that her doctor recommended that she use a combination of supplements and diet
rich in folate to increase folic acid intake. Six women had discussed folic acid with their
doctor during pregnancy and three women before pregnancy. None of the women
reported that their doctor had discussed folic acid with them during childbearing years.
Similar results have been reported by French et al., (2003). Most women reported that
their doctors discussed folic acid with them during their pregnancy at which time they
have already missed the opportunity to reduce the risk of NTD pregnancy. Although
some doctors discuss the issue before pregnancy giving women an opportunity to start
taking folic acid periconceptionally, doctors are still missing women who have unplanned
reported that only 4% of women in their study that had discussed folic acid with their
physician recalled having done so during the childbearing years. Perelman et al., (1996)
in a Toronto survey reported that as many as 40% of primary care doctors did not
mention periconceptional folic acid supplementation to women of childbearing age or
with those who were planning pregnancy. Only 17% of physicians recommended folate
to any woman of childbearing age and only 14% knew the appropriate timing for folate
supplementation. A recent survey reported by Gupta and Gupta (2000) from New Delhi,
India had similar results. They surveyed 30 practicing obstetricians and reported that 19
of them knew that folic acid could prevent birth defects. Even fewer (n=9) knew that it
should be taken before pregnancy and only 6 knew the correct dose of folic acid to be
taken. However, none of these obstetricians were prescribing periconceptional folic acid
supplementation or encouraging women to increase folate rich foods in their diet.
Another study examining the knowledge of 223 women's health care providers
(obstetricians, gynecologists and nurse-midwives) in Connecticut, Illinois, reported that
less than half of the health care providers surveyed (45%) recommended folic acid supplements to all female patients and many of them were unclear of the level of supplementation to be recommended (Pinkham and Cobb, 1999).

Physicians are considered credible sources of health information and clients are usually receptive to health messages provided by them. Bonin et al., (1998) indicated that educational information distributed by family physicians has the potential to be influential in bringing behavioral changes. In the current study as in other studies, physicians have been identified as the most popular preferred source of information for folic acid. Thus, doctors have the potential to be effective sources of nutrition information. However, a survey of nutrition knowledge of Canadian physicians in Alberta revealed that Canadian physicians need more training in nutrition (Temple NJ, 1999), although they did report that 98% of physicians surveyed by them correctly identified folate as the nutrient strongly associated with prevention of neural tube defects. With proper nutrition education, doctors have the potential to be an important messenger of information on folic acid and NTD to women of childbearing age.

In March 2002 Health Canada launched a public education campaign targeting doctors and other health professionals to increase the level of awareness and use of folic acid among women of childbearing age. However, media campaigns may be more effective in increasing the level of knowledge of folic acid and NTD for women of childbearing age who are less likely to visit their doctor to seek nutritional advice related to pregnancy.

It is important that the information and campaigns be carried out in Punjabi for most Sikh women. In our study almost 50% women were not fluent in English, and also
insisted that they would prefer to receive future information of folic acid and NTD in Punjabi. Thus educational efforts aimed at increasing the awareness and use of periconceptional folic acid supplementation among Sikh women must include strategies used for all women of childbearing age regardless of whether they are planning pregnancy or not and also regardless of their language preference/fluency.

6.4.2 Periconceptional Use of Folic Acid Supplement and NTD (Timing of supplement use and reasons why women didn’t take supplements prior to pregnancy)

In order for folic acid supplementation to be effective in reducing the risk of NTD it is imperative that the supplement be taken periconceptionally i.e. at least 3 months before pregnancy and in the first three months of pregnancy. Sikh women in the present study that had previously been pregnant (n=35) were asked if they had used a supplement before pregnancy and during pregnancy. The use of supplements during pregnancy was common (n=32; 91.4%), but before pregnancy was fairly low (n=5; 14.3%). All women consuming supplements during pregnancy were using prenatal vitamins upon their doctor’s advice. This increase in the use of supplements during pregnancy when compared to before pregnancy cannot be viewed as an intentional effort on the part of the women to increase folic acid consumption because most women in our study were unaware of the role of folic acid in reducing the risk of NTD affected pregnancy.

French et al., (2003) also reported that more women used a supplement containing folic acid, particularly prenatal vitamins, during gestation (89%) compared to before becoming pregnant (45%). However, compared to our study results, more women were consuming supplements before pregnancy in French’s study. The difference in study
population of the two studies may be responsible for the difference. French et al.'s population consisted of predominantly white women who were well educated in higher income groups compared to the Sikh women in our study. Also all of French et al.'s participants were fluent in English compared to only 50% women of our participants. Besides the difference in population, the difference in recruitment methods (random sample used by French et al., compared to the convenience sample used in this study) could also explain some of the difference.

In another study conducted at BC Women's Hospital, Morin et al., (2001) found that about 50% of study participants took folic acid containing vitamins prior to pregnancy. This is again higher than that of our study and the difference in population and the method of data collection may be responsible. The women in Morin's study were all fluent in English and were attending a genetic clinic. Besides they used a self-administered multiple choice type questionnaire unlike the interviewer administered open-ended questionnaire used by us.

Many studies have reported that the use of supplements is more common during pregnancy than it is before pregnancy (Bower et al., 1997; McGovern et al., 1999; Sayers et al., 1997; McDonnell et al., 1999). However, a higher proportion of women have reported consuming daily folic acid containing supplements prior to pregnancy in many countries in Europe and Australia. Public education campaigns and media campaigns in these countries may have played an important role in increasing the proportion of women using these supplements regularly.

The two most common reasons provided by Sikh women in the present study for choosing not to take a supplement prior to pregnancy was the belief that food alone
provides enough nutrients (n=15; 50%), followed by lack of awareness of the importance of folic acid (n=12; 40%). Lack of awareness was the most frequently identified reason for not taking a folic acid containing supplement prior to pregnancy (61%) by the predominantly white women in French et al.'s (2003) study, and it was followed by the belief that food alone supplies enough nutrients (33%). In Europe, McGovern et al., (1997) studied factors affecting the use of folic acid containing supplements among women of Glasgow who did not use supplements periconceptionally. Forty three percent reported lack of knowledge as the reason for not taking a supplement containing folic acid periconceptionally, 13% did not feel the need for it and 1% reported that it was expensive. In The Netherlands, de Jong-van den Berg et al., (1998) conducted a survey and reported that only 17% women in their study had taken folic acid supplements during the recommended periconceptional period. They reported that 46% women in their study said that they did not use supplements containing folic acid because they had not heard of the recommendation. The women also said that they did not take supplements because they did not like taking anything during pregnancy and/or that they did not think it was useful. All three Sikh women in the present study that did not use supplements containing folic acid during pregnancy reported that they felt sick/nauseated after taking them.

Thus, there seems to be a universal resemblance in the reasons identified by women for not taking supplement in the recommended periconceptional period. Educating women about the importance of folic acid and the right time to increase intake may overcome these barriers. In US, Europe and Australia where public education campaigns have been taking place for a few years now, there is a trend towards more women being aware of the role of folic acid in reducing NTD risk and also more women
using folic acid containing supplements during the recommended periconceptional period.

The first step in bringing about a behavioral change after the individual has been made aware of the relationship between folic acid and NTD is to identify their willingness to change. Results of the current study are encouraging as almost 90% (n=40) of the participants were willing to take a daily supplement of folic acid if it was demonstrated that folic acid reduced the risk of NTDs. Other studies where women were asked the same questions observed that approximately 70-78% of their participants were willing to take a daily supplement of folic acid (French et al., 2003; McGovern et al., 1997; Sayers et al., 1997). When the women were asked to indicate the preferred means of increasing their folate intake in the present study, 40% preferred to increase their folate intake through diet alone (n=18) and 47% (n=21) preferred to use both diet and supplement. Only 6 women (13%) reported that they would prefer to use only supplements to increase their folic acid intake. Although approximately 90% women in our study were willing to take a folic acid containing supplement, only 6 (13%) women said that they would prefer to use only supplements to increase their folic acid intake. Studies report that the Sikh population is less likely to consume supplements (Keena et al., 1987). Thus, it appears that if educational strategies were successful in increasing the knowledge and awareness of the relationship between folic acid, NTD affected pregnancies and the correct timing to take folic acid containing supplements, many women would be willing to take a daily supplement. Results from countries such as the Netherlands (de Jong-van den Berg, 1998), Ireland (Oleary et al., 2001; Sayers et al., 1997), Australia (Chan et al., 2001) and the US (Centres for Disease Control and
Prevention, 1999) have already shown that there is an increase in the proportion of women of childbearing age taking folic acid supplement following educational/media campaigns and this increase is concurrent with the increase in knowledge in these women. However, we face a potential barrier of language when dealing with women of ethnic population such as in the present study where, half of the study sample was not fluent in English. Efforts must therefore be made to accurately translate the information into Punjabi for it to be most effective in increasing the knowledge and awareness of Sikh women of childbearing age regarding folic acid and NTD risk and consequently increase the proportion of these women consuming a daily folic acid supplement.
6.5 Limitations of the Study

Although the current study is the first of its kind to look at folate status and knowledge of Sikh women of childbearing age in Canada and provides insight into their attitudes and perceptions regarding diet during pregnancy, these findings are subject to some limitations. The limitations are potentially due to the biases of recruitment methods, data collection methods and instruments and data management.

6.5.1 Subject Recruitment Methods

Since a database of Sikh population was not available to use, a random sampling method was not used for recruiting subjects in the current study. The subjects were recruited using posters, newspaper ads, a recruitment booth, and word of mouth. Thus, it is likely that we have recruited women that are obviously interested in nutrition and health. Also, since the women contacted us if they were interested in the study, we were unable to collect information regarding the characteristics of women that did not express an interest in the study. However, our study subjects had a range of education and income level and also we had equal distribution of women who were fluent in English or not. Thus, we may have avoided the potential of skewed recruitment due to these aspects.

Also, we recruited women of childbearing age, whether or not they were capable of becoming pregnant. It is possible that women who are capable of becoming pregnant are more aware of folate and are more likely to be using supplements compared to women that are not capable of becoming pregnant. Since information on the women’s capability of becoming pregnant was not collected, we were unable to make such comparisons. However, in our study we observed that most women said that they did not
use supplements prior to being pregnant, thus this limitation may not affect the results of proportion of women using folic acid supplements periconceptionally.

### 6.5.2 Data Collection Methods and Instruments

For the collection of dietary data and information on the knowledge and attitudes of women in the current study, we used self-report methods of questionnaire/interviews and thus we introduced the biases of memory and social desirability. The use of multiple (7) 24-hour dietary recalls using multiple pass methods reduced the potential of obtaining inaccurate information. Since the participant only had to recollect what she had consumed during the previous day the potential of inaccuracy due to memory is minimized. Also using 7 recalls over a period of one month including the representative number of weekdays and weekends added to the accuracy of the information collected. Accuracy of determining portion size is better if 3 dimensional food models are used, however, since we conducted our recalls over the phone, this was not possible. However, we compensated this by using the simple household measures such as measuring cups and spoons. There is also a possibility that participants may change their diet in order to appear socially desirable. In order to avoid this, the participants were kept unaware of the day they will be called to collect information of their previous day's dietary intake. Using multiple pass method for the collection of dietary information reduces the potential of interviewer induced bias and also the potential of omitting food items eaten.

The data collected during the in-person interview using the knowledge and attitudes questionnaire could have been prone to interviewer bias. In order to reduce this,
we used objective questions and the same interviewer collected information on all participants.

The other types of errors are those associated with data entry and the use of nutrient databases. Nutrient databases are not capable of providing exact measure of an individual’s nutrient intake, because they are not entirely complete (Thompson and Byers, 1994). Also, we used a database that was developed for the North American diet to analyze the diets of women whose diets comprise largely of ethnic food items. The database did not include exhaustive food composition data on all foods consumed by participants of the current study. Some foods such as the ‘Roti Atta’ flour used for making ‘rotis’ was not found in the database and thus information on the folate content of this product was obtained from the manufacturer and entered into the database for use in the analysis. Also, when a participant reported consumption of a mixed food dish, data on the individual ingredients of the mixed dish were requested from the participant and each ingredient was entered individually. Since folate is a heat labile nutrient, variations in the food storage, processing and preparation conditions may affect the folate levels of the food items. Thus, the actual folate levels consumed by the participants may be less than what is estimated by the database. Although this cannot be helped, the data-entry errors can be prevented. All data entered were checked three times before the data analysis was done.

Errors associated with the collection and analysis of blood samples can cause serious flaws in the estimation of red blood cell folate and plasma homocysteine levels. Using the same laboratory for the collection and analysis for all participants of the study reduced this error.
6.6 Generalizability of the Study Findings

Since we have not been able to collect information on the Sikh women that did not participate in the study, we are unable to say for sure if we have a population that is representative of Sikh women in Lower Mainland, BC. However, considering the fact that our study participant’s levels of education and income was evenly spread and also that we have approximately equal representation of women that are fluent in English and not, we could say that the results obtained from this study may be generalized to most Sikh women of childbearing age in BC. However, the availability of food items may vary from region to region in Canada. The amount of ethnic Indian foods available in the Lower Mainland, BC can be compared to the availability of Toronto and area, but this may not be true of certain other parts of the country such as the non-metropolitan cities and towns.

Since there are a limited number of studies done that looked at the diet of women of South Asian origin, it may be very tempting to extrapolate the results from this study to other women of South Asian origin such as all Indian women, or Pakistani, Bangladeshi and Sri Lankan women. However, we must note that the diets very between these countries and also within India among women from different states and within the same state between different religion and social classes.
6.7 Conclusion

This is the first study to examine the folate status and knowledge of Sikh women in Canada. This study also provides an insight into Sikh women's perceptions and attitudes towards diet during pregnancy. Most women in the study met the EAR from their dietary intakes and supplements combined, but only about a fourth of the participants were meeting the special recommendation for women capable of becoming pregnant. Folic acid fortification resulted in increasing the intake by 120 μg SFA/day, but did not help most women meet the special recommendation without the consumption of supplements containing folic acid. Only about half of the participants had red blood cell folate levels >906 nmol/L (level associated with reduced risk of NTD) and about a third of the participants had high plasma homocysteine levels (> 7.8 μmol/L). Close to half of the women had heard of folate and only a fraction of them were aware of the relation between folate and neural tube defects and the right time of taking the supplement to reduce the risk of NTD. Most women believed that diet played a role in pregnancy and indicated that they would change their diet during pregnancy and more than half of them believed in the concept of hot and cold foods.
6.8 Future Directions

6.8.1 Role of Physician

Physicians have been identified as the most desired source of information regarding folate and neural tube defects by Sikh women in the present study. Physicians are considered to be credible sources of information and thus work is needed to better inform physicians that care for Sikh women of childbearing age.

6.8.2 Impact of the New Media Campaign of Health Canada

Health Canada has launched a media campaign to increase the awareness of women regarding folate and neural tube defects and to encourage the women of childbearing age to consume a daily folic acid supplement of 400 μg SFA/day to reduce the risk of NTD. A study should be done to see if this campaign has affected all women’s knowledge and awareness in Canada, including the Sikh women.

6.8.3 $B_{12}$ Levels Assessment

It has been suggested that besides folate and homocysteine, $B_{12}$ levels may also play a role in the reduction of NTD risk. Also, it is suggested that folic acid fortification may mask the $B_{12}$ deficiency. Thus, a study must be designed to assess $B_{12}$ levels along with the red blood cell folate and plasma homocysteine levels.
6.8.4 Revisiting the Special Recommendation

The current study was unable to assess the effect of folic acid fortification on the red blood cell folate and plasma homocysteine levels of Sikh women, because prefortification levels were unavailable. Thus, it would be beneficial to see if fortification has been able to reduce the NTD prevalence/occurrence in Sikh women post fortification. Also, there are other studies that have indicated that folic acid fortification has helped to reduce the rate of NTD prevalence in other population groups. Thus, it may be worthwhile to revisit the special recommendation of 400 µg SFA/day, to assess the possibility that it may be set too high.
References:


National Health and Medical Research Council; Revised statement on the relationship between dietary folate and neural tube defects such as spina bifida. 115th Session, Canberra, 1995.


Spina bifida and Hydrocephalus. [www.sbhac.ca/sbenbro.html]


Taking folic acid before you get pregnant. Motherisk Recommendations. Web page of The Hospital for Sick Children. [www.motherisk.org/recomm/folic.htm]


APPENDIX 2

LETTER TO INSTITUTES FOR MAIN STUDY SUBJECT RECRUITMENT

DIETARY INTAKES AND NUTRITION KNOWLEDGE OF SIKH WOMEN OF
CHILD BEARING AGE IN BRITISH COLUMBIA

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   Department of Human Nutrition
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   Contact Phone # - 822-6869

Co-Investigator: Mrs. Dipika Desai
   Department of Human Nutrition
   U.B.C.
   Contact Phone # - 822-6869
   Graduate Student (MSc) in Human Nutrition

We would like to invite women in your institution who are between the ages of 18-45
years, non-pregnant or lactating, and Sikh to participate in a research study to determine
the dietary intakes of Sikh women of child bearing age in British Columbia. The purpose
of this project is to determine the daily nutrient intakes of these women and to compare
and correlate it with the blood levels of the nutrients. The project also proposes to assess
the knowledge and awareness of nutrition recommendations for non-pregnant women of
childbearing age in British Columbia, and to study the dietary habits of these women.

The last provincial nutrition survey was conducted in the early seventies as part of the
Nutrition Canada Survey. Presently, we have no evidence of dietary intake, nutritional
status, and awareness to indicate if non-pregnant women are consuming adequate
amounts of nutrients. Also, there is little information about the dietary habits of Sikh
women of childbearing age residing in British Columbia.

To participate in this study the woman must be non-pregnant and between the ages of 18-
45 years. Women who have been pregnant or lactating within previous six months will be
excluded from the study. We would like to post a flyer, inviting the eligible women for
participating, on your bulletin board. A copy of the flyer is attached herewith. The
prospective participant can call the researcher at the contact number on the flyer. If the
woman agrees to participate in the study, the researcher will arrange an interview at the
participant's home at her convenience.

The time required for the interview is one hour. Before the interview begins, the woman
will have to sign a written informed consent. All information will be kept confidential.
During the home visit the woman will be interviewed regarding her daily food intake using a food frequency questionnaire and a 24-hour recall method. The second part of the interview will seek some personal background information as well as information regarding the woman's dietary practices and awareness of nutrition recommendations and related health issues. The woman's participation in this study is strictly voluntary. Please be aware that the woman may refuse to participate or withdraw from the study at any time.

Any information resulting from this study will be kept strictly confidential. All documents will be identified only by code number and kept in a locked filing cabinet. Participants will not be identified by name in any reports of completed study.

We hope that you will find this a worthwhile project to be involved with. Your participation and the participation of your clients, in this study will be greatly valued. With your help, the information gathered will help health educators in designing future educational campaigns. In return for the participation, we would like to provide the women with a written copy of their dietary analysis and two nutrition education pamphlets.

We look forward to speaking with you and your clients.

Sincerely

Ryna Levy Milne   Dipika Desai
APPENDIX 4

INFORMED CONSENT FOR INDIVIDUALS BEING INTERVIEWED

DIETARY INTAKES AND NUTRITION KNOWLEDGE OF PUNJABI WOMEN OF CHILD BEARING AGE IN BRITISH COLUMBIA

Principal Investigator: Dr. Ryna Levy Milne, Ph.D., RDN
Assistant Professor
Food, Nutrition and Health
U.B.C.
Contact Phone # - 822-6869

Co-Investigator: Mrs. Dipika Desai
Food, Nutrition and Health
Graduate Student (M.Sc) in Human Nutrition
U.B.C.
Contact Phone # - 822-6869

Purpose:

The purpose of this project is to determine the daily nutrient intakes of the Punjabi women of childbearing age in British Columbia and to compare and correlate it with the blood levels of the nutrients. The project also proposes to assess the knowledge and awareness of nutrition recommendations for non-pregnant women of childbearing age in British Columbia, and to study the dietary habits of these women. The last provincial nutrition survey was conducted in the early seventies as part of the Nutrition Canada Survey. Presently, we have no evidence of dietary intake, nutritional status, and awareness to indicate if non-pregnant women are consuming adequate amounts of nutrients. Also, there is little information about the dietary habits of Punjabi women of childbearing age residing in British Columbia.

Study Procedures:

If you agree to participate in the study you will be asked to participate in two 30-minute interviews in your home. During the home visit you will be interviewed regarding your daily food intake using a 24-hour recall method. The second interview will seek some personal background information as well as information regarding your dietary practices and awareness of nutrition recommendations and related health issues. You will also participate in six 24-hour recalls over the phone in between the two in-home interviews. The complete study will span over a period of 30 days.

To learn more about your nutritional status, we ask that you agree to have a blood sample taken by a laboratory technician at the BC Biomedical Laboratories. At the time of the second in-home interview, the research assistant will provide you with a requisition to
take to the laboratory closest to your home to have blood sample taken. The amount of blood taken will be 2 ml (1/2 teaspoon). Side effects of having blood samples taken include mild pain at the site and possible minor bruising. A copy of the results of the blood work will be sent to you by mail.

Confidentiality:

Any information resulting from this study will be kept strictly confidential. All subject names will be coded and protected by use of a study number and documents will be kept in locked filing cabinet. You will not be identified by name in any reports of the completed study. Data records will be kept on computer hard drive (accessed by password) and on floppy disk (stored in locked filing cabinet).

Contact:

If you have any questions or desire further information with respect to this study, you may contact Dr. Ryna Levy Milne or her associate at (604)822-6869.

If you have any concerns about your treatment or rights as a research subject, you may contact the Director of Research Services at the University of British Columbia, Dr. Richard Spatley, at (604)822-8598.

Consent:

You understand that your participation in this study is entirely voluntary and that you may refuse to participate or you may withdraw from the study at any time.

You have received a copy of this consent form for your own records.

You consent to participate in this study.

Subject Signature __________________________ Date __________

Signature of Witness __________________________ Date __________
APPENDIX 5

DEVELOPMENT OF THE WOMEN'S KNOWLEDGE AND BEHAVIORS QUESTIONNAIRE

As, there was no valid FFQ developed for the assessment of food and/or nutrient intake in the Sikh or Punjabi population in India or elsewhere, a preliminary FFQ was developed, adapted from a validated FFQ designed to determine the folate intake of women of childbearing age in the Lower Mainland (French et al., 2001). To the original food list, some commonly consumed ethnic food items like ‘roti’, ‘paratha’, and ‘paneer’ were added. The ethnic foods were identified from previous dietary studies (Jain et al., 1999) and discussions with Sikh women in the community. Focus groups were arranged with women similar to the target population, to discuss this preliminary FFQ, and to identify more ethnic food items (that have the potential to contribute to folate intake) consumed by this population. The discussion was also used to learn if the Sikh women were familiar with the foods already in the food list. The final FFQ is presented in Appendix 6.

The new culturally relevant FFQ was tested for validity with 20 Sikh women between the ages of 18 and 45 years, who were fluent in English and residing in the Lower Mainland. The women were recruited by posting flyers on community centres’ bulletin boards and on the campus of the University of British Columbia. The FFQ was verbally administered to each participant requesting the participant to report their consumption pattern from the previous six months and using food models to estimate portion size. These women were then instructed to record seven days of food (Appendix 6) and beverage intake (including a representative number of weekdays and weekend days) over a period of four weeks as suggested by Firth et al. (1998).
Data from the FFQ and 7-day dietary records were entered into the Food Processor II Program (Version 7.22, Database Revised April 1998, ESHA Research, Salem, Oregon) for analysis. The folate values for food items affected by the mandatory folic acid fortification program that became effective in 1998 were obtained from the American database since this version of Food Processor had updated American nutrient values to reflect the fortification of bread and grain products, but not the Canadian values. This FFQ would be considered a valid instrument for assessing the folate intake of the targeted group if the correlation coefficient between the FFQ and 7-day dietary records was between 0.4 and 0.7 (Thompson and Byers, 1994). The mean folate intakes obtained from both the methods were compared using paired samples t-tests to make sure that mean folate intakes as analyzed by both the methods were similar.

The mean folate intake by the FFQ was 508.2 ± 111.7 µg/day and by the 7-day dietary records was 348.6 ± 78.8 µg/day. The folate intakes estimated by the two methods tended to correlate (r=0.460; p=0.073) but the mean folate intakes from these methods were significantly different (difference of means = 159.56 µg, p=0.000). Based on these results the FFQ was not considered a valid instrument to assess the individual folate intakes of our target population.
SEVEN DAY FOOD RECORD

ID #:______

Dear Participant,

Please record everything you eat and drink on forms attached, for seven days over the next four weeks. Please include five weekdays and two weekend days.

Please read the instructions below before beginning to record your food intake:

1. DON’T change your eating habits during the days that you record your intake.
2. Write EVERYTHING THAT YOU EAT AND DRINK (including alcohol, but excluding water). Be sure to include all snacks eaten between meals (e.g. on the desk while working or while watching TV etc…). Record immediately after each meal and snack. (You may take this form to work with you, if you work outside your home)
3. In the first column, record the time you consumed the food you are talking of.
4. In the second (middle) column, write the name of food item and describe the brand name, contents of mixed dishes (as and when applicable).
5. In the third (right hand side) column, write the amount and/or size of the food.
6. Use VOLUME measures (cups, teaspoons, tablespoons, milliliters) for cereals, rice, pasta, vegetables, ‘sabjis’, salads, canned or sliced/cut fruits, beverages, peanut butter, salad dressings, margarine, butter, oil, chutneys, indian pickles, sauces, soups, gravies, ‘dals’, sugar, honey, jam, etc.
7. Use WEIGHTS (grams, ounces) where possible for meats and cheeses

SAMPLE:

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<tr>
<th>Time</th>
<th>Food</th>
<th>Amount</th>
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<td>Breakfast</td>
<td>Aloo Paratha: Roti flour, boiled potatoes, spices. Pan fried in oil</td>
<td>1 (6 inch diameter)</td>
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<td></td>
<td>Tea with milk and spices</td>
<td>1 cup</td>
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<td>Lunch</td>
<td>Vegetarian Sandwich: White Bread</td>
<td>2 slices</td>
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<td>Lettuce leaf</td>
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<td>Tomato slices</td>
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<td>Coriander chutney</td>
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<td>Milk (Homo)</td>
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<td>Snack</td>
<td>Apple</td>
<td>1 small</td>
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FOOD FREQUENCY QUESTIONNAIRE FOR THE SIKH WOMEN OF CHILDBEARING AGE

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PRELIMINARY KNOWLEDGE AND ATTITUDES QUESTIONNAIRE (SIKH WOMEN)

1. Do you think that your diet can influence your pregnancy outcome?

2. Do you change your diet during pregnancy? Who would advice you to change or not to change your diet?

3. Do you avoid eating any food during pregnancy?

4. If yes, then what do you avoid and during what period of pregnancy?

5. Do you add any new food to your diet during pregnancy that would otherwise not be eaten by you, or be rarely eaten?

6. If yes, what do you add and during what period of pregnancy?

7. Do you believe in the concept of “hot” and “cold” foods?

8. If yes, can you give me examples of both “hot” and “cold” foods?

9. Have you ever heard of folic acid, also called as folacin or folate?

10. Can you tell me what you think folic acid is?

11. Where did you hear about folic acid?

12. Are you aware of a relationship between folic acid and your health?

13. If so, can you tell me what you think folic acid does?

14. During what period of time in a woman’s life do you think that she should take folic acid supplements to decrease the risk of birth defects?

15. Do you think that you are getting enough folic acid from your diet?

16. Can you name a few good sources of folic acid?

17. Have you ever been pregnant in the past?

18. If so, did you take nutritional supplements before pregnancy? If yes, what type?
19. If no, why did you choose not to take supplements?

20. Did you take nutritional supplements during pregnancy? If yes, what type?

21. If no, why did you choose not to take supplements?

22. Have you heard of neural tube defects or spina bifida?

23. If yes, do you know what spina bifida is?

24. To receive more information regarding folic acid and the prevention of birth defects, where do you think Sikh women would like to get this information from?

25. Would you be willing to take a supplement containing folic acid everyday throughout your childbearing years if it was proven that the supplement could reduce the chances of having a child with certain birth abnormalities such as spina bifida?

26. Can you think of any reasons why a woman would not take a daily supplement of folic acid during the childbearing years if she knew that such a supplement could reduce the risk of birth defects?

27. Some of the foods contain added vitamins and minerals. Folic acid is one such nutrient that is added to some foods. Can you list the foods to which it is added?

28. Has your doctor ever discussed getting enough folic acid with you?

29. If yes, how did your doctor recommend that you get enough folic acid?

30. When did your doctor tell you that it was important to get enough folic acid?
INFORMED CONSENT FOR THE FOCUS GROUP DISCUSSIONS

DEVELOPMENT OF RESEARCH TOOLS TO DETERMINE DIETARY INTAKES AND NUTRITION KNOWLEDGE OF SIKH WOMEN OF CHILD BEARING AGE IN BRITISH COLUMBIA

Principle Investigator: Dr. Ryna Levy Milne, Ph.D., RDN
Assistant Professor
Department of Human Nutrition
U.B.C.
Contact Phone # - 822-6869

Co-Investigator: Mrs. Dipika Desai
Department of Human Nutrition
U.B.C.
Contact Phone # - 822-6869
Graduate Student (MSc) in Human Nutrition

Purpose:

The purpose of this project is to develop a valid food frequency questionnaire in order to determine the daily nutrient intakes of the Sikh women of childbearing age in British Columbia and to develop a survey to assess the knowledge and awareness of nutrition recommendations for non-pregnant women of childbearing age in British Columbia. The last provincial nutrition survey was conducted in the early seventies as part of the Nutrition Canada Survey. Presently, we have no evidence of dietary intake, nutritional status, and awareness to indicate if non-pregnant women are consuming adequate amounts of nutrients. Also, there is little information about the dietary habits of Sikh women of childbearing age residing in British Columbia. Since the Sikh population consumes a lot of ethnic food items, it is important to develop a culturally relevant food frequency questionnaire.

Study Procedures:

If you agree to participate in the study, you will attend the one hour focus group discussion at the institute. During the focus group we/you will discuss their dietary practices and awareness of nutrition recommendations and related health issues. Some personal information such as age, occupation, education, will be asked for.
Confidentiality:

Any information resulting from this study will be kept strictly confidential. All subject names will be coded and protected by use of a study number and documents will be kept in locked filing cabinet. You will not be identified by name in any reports of the completed study. Data records will be kept on computer hard drive (accessed by password) and on floppy disk (stored in locked filing cabinet).

Contact:

If you have any questions or desire further information with respect to this study, you may contact Dr. Ryna Levy Milne or her associate at (604)822-6869.

If you have any concerns about your treatment or rights as a research subject, you may contact the Director of Research Services at the University of British Columbia, Dr. Richard Spatley, at (604)822-8598.

Consent:

You understand that your participation in this study is entirely voluntary and that you may refuse to participate or you may withdraw from the study at any time.

You have received a copy of this consent form for your own records.

You consent to participate in this study.

__________________________  ______________________
Subject Signature Date

__________________________  ______________________
Signature of Witness Date
LETTER TO INSTITUTES FOR FOCUS GROUP SUBJECT RECRUITMENT

DEVELOPMENT OF RESEARCH TOOLS TO DETERMINE DIETARY INTAKES AND NUTRITION KNOWLEDGE OF SIKH WOMEN OF CHILD BEARING AGE IN BRITISH COLUMBIA

Principle Investigator: Dr. Ryna Levy Milne, Ph.D., RDN
   Assistant Professor
   Department of Human Nutrition
   U.B.C.
   Contact Phone # - 822-6869

Co-Investigator: Mrs. Dipika Desai
   Department of Human Nutrition
   U.B.C.
   Contact Phone # - 822-6869
   Graduate Student (MSc) in Human Nutrition

We would like to invite women in your institution who are between the ages of 18-45 years, non-pregnant or lactating, and Sikh to participate in a research study to determine the dietary intakes of Sikh women of child bearing age in British Columbia. The purpose of this project is to develop a valid food frequency questionnaire in order to determine the daily nutrient intakes of these women and to develop a survey to assess the knowledge and awareness of nutrition recommendations for non-pregnant women of childbearing age in British Columbia.

The last provincial nutrition survey was conducted in the early seventies as part of the Nutrition Canada Survey. Presently, we have no evidence of dietary intake, nutritional status, and awareness to indicate if non-pregnant women are consuming adequate amounts of nutrients. Also, there is little information about the dietary habits of Sikh women of childbearing age residing in British Columbia. Since the Sikh population consumes a lot of ethnic food items, it is important to develop a culturally relevant food frequency questionnaire.

To participate in this study the woman must be non-pregnant and between the ages of 18-45 years. Women who have been pregnant or lactating within previous six months will be excluded from the study. We would like to post a flyer, inviting the eligible women for participating, on your bulletin board. A copy of the flyer is attached herewith. The prospective participant can call the researcher at the contact number on the flyer. If the woman agrees to participate in the study, she will attend the focus group discussion at the institute, with your permission, and at a time most convenient for the institute, the women, and the researcher.
The time required for the focus group discussion is one hour. Before the discussion begins, the women will have to sign a written informed consent. All information will be kept confidential.

During the focus group the women will discuss their dietary practices and awareness of nutrition recommendations and related health issues. The woman's participation in this study is strictly voluntary. Please be aware that the woman may refuse to participate or withdraw from the study at any time.

Any information resulting from this study will be kept strictly confidential. All documents will be identified only by code number and kept in a locked filing cabinet. Participants will not be identified by name in any reports of completed study.

We hope that you will find this a worthwhile project to be involved with. Your participation and the participation of your clients, in this study will be greatly valued. With your help, the information gathered will help health educators in designing future educational campaigns.

We look forward to speaking with you and your clients.

Sincerely

Ryna Levy Milne    Dipika Desai
APPENDIX 11

KNOWLEDGE AND ATTITUDES QUESTIONNAIRE AND RECORDING FORM
Part A: Knowledge and attitudes questionnaire (Sikh women)

1. Do you think that your diet can influence your pregnancy outcome?
   1. Yes
   2. No
   3. Not sure

2. Do you change your diet during pregnancy?
   1. Yes
   2. No
   3. Not sure

*If no to #2 skip to #7.

3. Who would advice you to change or not to change your diet?
   1. Mother
   2. Mother-in-law
   3. Other relatives
   4. Friends
   5. Doctor
   6. Media
   7. Others

*Participant is allowed more than one response to this question, please probe for additional information until the respondent stops providing information.

4. Do you avoid eating any food during pregnancy?
   1. Yes
   2. No

*If no to #4 skip to #6

5. If yes, then what do you avoid and during what period of pregnancy?

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<thead>
<tr>
<th>Food</th>
<th>Trimester (1st, 2nd, 3rd)</th>
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*Participant is allowed to select more than one trimester.
6. Do you add any new food to your diet during pregnancy that would otherwise not be eaten by you, or be rarely eaten?
   1. Yes
   2. No

If no to #6, skip to #8.

7. If yes, what do you add and during what period of pregnancy?

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<thead>
<tr>
<th>Food</th>
<th>Trimester (1\text{st}, 2\text{nd}, 3\text{rd})</th>
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*Participant is allowed to select more than one trimester.*

8. Do you believe in the concept of "hot" and "cold" foods?
   1. Yes
   2. No

If no to #8, skip to #10.

9. If yes, can you give me examples of both "hot" and "cold" foods?

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<th>Food</th>
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10. Have you ever heard of folic acid, also called as folacin or folate?
    1. Yes
    2. No

If no to #10, please skip to #18.

11. Can you tell me what you think folic acid is?
1. Vitamin
2. Some thing needed for pregnancy to help you have a healthy baby
3. Something that prevents birth defects
4. Something similar to iron
5. Nutrient
6. Miscellaneous
7. Don’t know

12. Where did you hear about folic acid?
   1. Family physician
   2. Magazines/news papers
   3. Television shows/radio
   4. Internet
   5. Spouse/partner/family
   6. Friends
   7. School
   8. Educational pamphlets
   9. Other health professionals (public health nurse, pharmacist, RDN, etc...)
   10. Others

   *participant is allowed more than one response to this question, please probe for additional information until the respondent stops providing information.

13. Are you aware of a relationship between folic acid and your health?
   1. Yes
   2. No

If no to #13, please Skip to #17

14. If so, can you tell me what you think folic acid does?
   1. Prevention of birth abnormalities by adequate levels of intake
   2. Association with cardiovascular disease
   3. Association with cervical and/or colon cancer
   4. Vitamin
   5. Other( eg. Megaloblastic anemia)
   6. Don’t know
   7. Incorrect response

   *participant is allowed more than one response to this question, please probe for additional information until the respondent stops providing information.

If the participant responds “1” to question 14, go to #15.
If the participant has any other response, please go to #16.

15. During what period of time in a woman’s life do you think that she should take folic acid supplements to decrease the risk of birth defects?
1. During childbearing years
2. When planning pregnancy (before pregnancy)
3. During pregnancy
4. Don't know
5. Incorrect response

16. Do you think that you are getting enough folic acid from your diet?
   1. Yes
   2. No
   3. Don’t know

17. I am going to ask you whether you think that following foods are good sources of folic acid or not. Please tell me if you think that the food is a good source of folic acid by saying “yes”. If you think that it is not a good source of folic acid, please answer by saying “no” and if you are not sure whether the food is a good source of folic acid, please say “don’t know”.
   • Oranges/orange juice
   • Beef
   • Chocolate
   • Asparagus
   • Broccoli
   • Grapes
   • Breakfast cereals
   • Garlic
   • Dried beans and peas

18. Have you ever been pregnant in the past?
   1. Yes
   2. No

If never been pregnant before, please skip to question #23.

19. Did you take nutritional supplements before your pregnancy? If so, what type of supplement did you take?
   1. Folic acid supplements
   2. General multivitamin
   3. Prenatal vitamin
   4. Didn’t take supplements

*If she didn’t take supplements, please go to question #20.
If she did take supplements, please go to question #21.*

20. If no, why did you choose not to take supplements?
   1. Didn’t know that it was important to take
   2. Financial expense
   3. Too busy/change of normal routine

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4. Don’t believe recommendations/don’t care about health/nutrition
5. Doctor didn’t inform me
6. Food sources provide enough
7. Other (please note __________ )
8. Don’t know

*permitted to choose more than one answer to this question

21. Did you take nutritional supplements during pregnancy? If yes, what type?
   1. Folic acid supplements
   2. General multivitamin
   3. Prenatal vitamin
   4. Didn’t take supplements

If she didn’t take supplements, please go to question #22
If she did take supplements, please go to question #23

22. If no, why did you choose not to take supplements?
   1. Didn’t know that it was important to take
   2. Financial expense
   3. Too busy/change of normal routine
   4. Don’t believe recommendations/don’t care about health/nutrition
   5. Doctor didn’t inform me
   6. Food sources provide enough
   7. Other (please note __________ )
   8. Don’t know

*permitted to choose more than one answer to this question

23. Have you heard of neural tube defects or spina bifida?
   1. Yes
   2. No

If never heard of NTD/SB, please skip to Bridge 1 and question #25

24. If yes, do you know what spina bifida is?
   1. A defect that you are born with
   2. A condition that develops later in life
   3. Don’t know
   4. Spinal/NT problem
   5. Other

Bridge 1:
So far in this survey, I’ve mentioned folic acid and birth abnormalities. Folic acid is a B vitamin that helps prevent birth abnormalities called neural tube defects such as spina bifida. Some research has shown recently that getting enough folic acid can
decrease the chances of having a child affected with birth abnormalities such as spina bifida by up to 50%. I'd like to ask you some questions about how you feel about increasing folic acid intake by using a supplement everyday to decrease the risk of having a child born with a birth abnormality."

25. To receive more information regarding folic acid and the prevention of birth defects, where do you think Sikh women would like to get this information from?
   1. Family
   2. Friends
   3. Family physician
   4. Pamphlets
   5. Television programs/radio
   6. Newspapers/magazines/books
   7. Food labels
   8. Package inserts such as in boxes of feminine hygiene products
   9. Pharmacist
   10. Health food store staff
   11. Media campaign (eg. Ads, billboards, etc)
   12. Nutritionist
   13. Other

*participant will be given a card with potential answers on it and asked to choose the ones that are most relevant to them. Please rotate the use of cards. Continue probing until the woman gives no further responses.

26. Would you be willing to take a supplement containing folic acid everyday throughout your childbearing years if it was proven that the supplement could reduce the chances of having a child with certain birth abnormalities such as spina bifida?
   1. Yes
   2. No
   3. Need more information before making a decision
   4. Don't know

27. To increase your intake of folic acid, would you rather take a folic acid supplement or increase your intake of foods that are good sources of folate?
   1. Folic acid supplement
   2. Change dietary habits
   3. Both
   4. Don't know

28. Can you think of any reasons why a woman would not take a daily supplement of folic acid during the childbearing years if she knew that such a supplement could reduce the risk of birth defects?
   1. No reasons not to take a supplement
   2. Financial expense
   3. Too busy/change in normal routine
4. Don’t believe recommendations/don’t care about nutrition or health
5. Don’t believe in taking supplements
6. Need more information before committing to taking supplement
7. Believe that food sources provide adequate amounts so supplements not needed
8. Don’t know

*participant allowed to choose more than one response to this question
*continue to probe until the participant gives no further information

29. Some of the foods contain added vitamins and minerals. Folic acid is one such nutrient that is added to some foods. I am going to list several foods and would like you to tell me if you think that these foods have folic acid added to them. Please answer yes or no.
   1. Bread
   2. Apple juice
   3. Rice
   4. Pasta
   5. Milk
   6. Margarine
   7. Breakfast cereals

For the following question if the woman has indicated earlier in the survey that she has received information from her doctor about folic acid, please lead into the question by saying “You’ve mentioned that you heard about folic acid from your family physician…” (Trying to emphasize the difference between telling them about folic acid and actually providing details about how and when to get enough.)

30. Has your doctor ever discussed getting enough folic acid with you?
   1. Yes
   2. No

If no, please skip to demographics survey

31. If yes, how did your doctor recommend that you get enough folic acid?
   1. Increase intake through supplement containing folic acid
   2. Increase intake by eating folate rich foods
   3. Both through the use of supplements and folate rich food
   4. Didn’t tell me how

32. When did your doctor tell you that it was important to get enough folic acid?
   1. During child bearing years
   2. Before pregnancy
   3. During pregnancy
   4. Other
Knowledge and attitudes questionnaire (Sikh women)

Part B: Demographic Survey

Now, I’d like to ask you some questions that will help us characterize the women participating in the study. Just to remind you again, your name does not appear on any of these forms and all answers will remain confidential.

1. When is your birthday?  
   YYYY

2. What is your marital status?  
   01 married/common-law  
   02 divorced  
   03 widowed  
   04 single

3. Do you have any children?  
   01 yes  
   02 no

   If no, please go to question #6.  
   If yes, please go to question #4.

4. IF yes to #3, what is the date of your last child’s birth?  
   YYYY/MM/DD

5. Were any of your children born with a birth defect? IF yes, what type of birth defect?  
   01 folate related  
   02 other  
   03 no

6. Have you ever had a miscarriage?  
   01 yes  
   02 no

   If yes, please go to question #7.  
   If no, please go to question #8.

7. How many miscarriages have you had?  
   XXX

8. Are you taking any vitamin/mineral supplements?  
   01 multivitamin  
   02 prenatal vitamin  
   03 folic acid  
   04 specific nutrients
9. Please record the amount of folic acid in the supplement used.

10. During the past 6 months how often would you say that you took these supplements?
    01 not regularly
    02 _______ times per week
    03 _______ times per day

11. Are you a vegetarian?
    01 vegan
    02 other type of vegetarian
    03 no

12. Do you smoke cigarettes? If yes, how many per day?
    01 1-5 cigarettes
    02 6-10 cigarettes
    03 11-20 cigarettes
    04 >20 cigarettes
    05 non-smoker

13. Do you consume alcoholic drinks? If yes, how many per day?
    01 1-2
    02 3-6
    03 >6
    04 non-drinker

14. Are you Sikh by birth?
    01 yes
    02 no

15. How many years have you lived in Canada?
    01 <1 year
    02 1-5 years
    03 6-10 years
    04 >10 years

16. What is the highest level of education that you have completed?
    01 less than grade 8
    02 completed high school
    03 completed post-secondary college/university

17. What is your annual household income?
    01 less than 20,000
    02 21,000-30,000
    03 31,000-40,000
18. How many people live in your household?
   XXXX
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<th>2 no</th>
<th>3 ns</th>
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<td>2 no</td>
<td>3 ns</td>
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<td>2 mother-in-law</td>
<td>3 other relative</td>
<td>4 friends</td>
<td>5 doctor</td>
<td>6 media</td>
<td>7 others</td>
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<tr>
<td>KQ. 8</td>
<td>Believe on/off foods?</td>
<td>1 yes</td>
<td>2 no</td>
<td></td>
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<td></td>
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<td>KQ. 9</td>
<td>List</td>
<td></td>
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<tr>
<td>KQ. 10</td>
<td>Heard about folate?</td>
<td>1 yes</td>
<td>2 no</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>KQ. 11</td>
<td>What is Folate?</td>
<td>1 vitamin</td>
<td>2 need for preg</td>
<td>3 prevent birth de.</td>
<td>4 like iron</td>
<td>5 nutrient</td>
<td>6 misc</td>
<td>7 don't know</td>
</tr>
<tr>
<td>KQ. 12</td>
<td>Where hear about folate?</td>
<td>1 doctor</td>
<td>2 mags/papers</td>
<td>3 tv/radio</td>
<td>4 internet</td>
<td>5 spouse/family</td>
<td>6 friends</td>
<td>7 school</td>
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<tr>
<td></td>
<td></td>
<td>8 pamph</td>
<td>9 health prof.</td>
<td>10 others</td>
<td></td>
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<tr>
<td>KQ. 13</td>
<td>Aware folate/health</td>
<td>1 yes</td>
<td>2 no</td>
<td></td>
<td></td>
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<tr>
<td>KQ. 14</td>
<td>Relations hip bet fola &amp; hlth</td>
<td>1 prevent birth defects</td>
<td>2 associatio n with cvd</td>
<td>3 cervical colon cancer</td>
<td>4 vitamin</td>
<td>5 other</td>
<td>6 don't know</td>
<td>7 misc</td>
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<td>KQ. 15</td>
<td>Period to take folate</td>
<td>1 child-bearing years</td>
<td>2 before pregnanc y</td>
<td>3 during pregnanc y</td>
<td>4 don't know</td>
<td>5 misc</td>
<td></td>
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<td>KQ. 16</td>
<td>Diet enough.</td>
<td>1 yes</td>
<td>2 no</td>
<td>3 dk</td>
<td></td>
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<td>KQ. 17</td>
<td>Folate food sources</td>
<td>Orange 1 2 3 y n d</td>
<td>Beef 1 2 3 y n d</td>
<td>Chocol 1 2 3 y n d</td>
<td>Aspara 1 2 3 y n d</td>
<td>Broccol 1 2 3 y n d</td>
<td>Grapes 1 2 3 y n d</td>
<td>Cereals 1 2 3 y n d</td>
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<td></td>
<td>Garlic 1 2 3 y n d</td>
<td>Bns/pea 1 2 3 y n d</td>
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<td>y</td>
<td>2</td>
<td>n</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<td>folic acid</td>
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<td>prenatal vitamin</td>
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<td>Why not before?</td>
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<td>didn’t know</td>
<td>2</td>
<td>finances</td>
<td>3</td>
<td>busy/change</td>
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<tr>
<td>Suppl during preg?</td>
<td>1</td>
<td>folic acid</td>
<td>2</td>
<td>multivitamin</td>
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<td>prenatal vitamin</td>
<td>4</td>
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<td>KQ. 22</td>
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<td>2</td>
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<td>folic acid</td>
<td>2</td>
<td>multivitamin</td>
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<td>prenatal vitamin</td>
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<td>KQ. 23</td>
<td>Heard of SB?</td>
<td>1</td>
<td>y</td>
<td>2</td>
<td>n</td>
<td>3</td>
<td>4</td>
<td>5</td>
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<tr>
<td>KQ. 24</td>
<td>What is SB?</td>
<td>1</td>
<td>born with</td>
<td>2</td>
<td>develop later</td>
<td>3</td>
<td>don’t know</td>
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<td>KQ. 25</td>
<td>Sources of more info?</td>
<td>1</td>
<td>family</td>
<td>2</td>
<td>friends</td>
<td>3</td>
<td>family doctor</td>
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<td>package inserts</td>
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<td>pharmacist</td>
<td>10</td>
<td>hth fd store</td>
<td>11</td>
<td>media campaign</td>
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<td>KQ. 26</td>
<td>Will tk daily suppl?</td>
<td>1</td>
<td>yes</td>
<td>2</td>
<td>no</td>
<td>3</td>
<td>more info</td>
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<td>KQ. 27</td>
<td>Supplem ent or food</td>
<td>1</td>
<td>supplement</td>
<td>2</td>
<td>food</td>
<td>3</td>
<td>both</td>
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<td>Why not take suppl?</td>
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<td>no reason</td>
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<td>2</td>
<td>multivitamin</td>
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<td>prenatal vitamin</td>
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<td>KQ. 29</td>
<td>Fortification</td>
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<td>Bread</td>
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<td>Applejack</td>
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<td>Rice</td>
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<td></td>
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<td>1</td>
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<td>n</td>
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<td>d</td>
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<td>5</td>
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<tr>
<td>KQ. 31</td>
<td>How tell to increas</td>
<td>1</td>
<td>suppleme nt</td>
<td>2</td>
<td>food sources</td>
<td>3</td>
<td>suppl/food</td>
<td>4</td>
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<tr>
<td>KQ. 32</td>
<td>When to increas</td>
<td>1</td>
<td>childbearing</td>
<td>2</td>
<td>before preg</td>
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### Part B:

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<td>DQ. 1</td>
<td>Birth year</td>
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<td>DQ. 2</td>
<td>Marital status</td>
<td>1</td>
<td>married</td>
<td>2</td>
<td>divorced</td>
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<td>DQ. 3</td>
<td>Children?</td>
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<td>yes</td>
<td>2</td>
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<td>DQ. 4</td>
<td>Last child birthday</td>
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<td>DQ. 5</td>
<td>Any child w/birth def</td>
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<td>folate rltl</td>
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<td>DQ. 6</td>
<td>Ever had miscar?</td>
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<td>DQ. 7</td>
<td># miscar</td>
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<td>DQ. 8</td>
<td>Vit/min suppl</td>
<td>1</td>
<td>multivit</td>
<td>2</td>
<td>folate</td>
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<td>DQ. 9</td>
<td>Amount of folate?</td>
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<td>DQ. 10</td>
<td>Frequency of vit?</td>
<td></td>
<td>Not regularly</td>
<td>X/day:</td>
<td>X/week:</td>
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<td>DQ. 11</td>
<td>Are you a vegetarian?</td>
<td>1</td>
<td>vegan</td>
<td>2</td>
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<td>DQ. 12</td>
<td>Do you smoke?</td>
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<td>yes</td>
<td>2</td>
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<td>Alcohol?</td>
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<td>Sikh by birth?</td>
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<td>yes</td>
<td>2</td>
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<td>Years in Canada?</td>
<td>1</td>
<td>&lt; 1 yr</td>
<td>2</td>
<td>1-5 yr</td>
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<td>DQ. 16</td>
<td>Highest level of education</td>
<td>1</td>
<td>&lt; gr 8</td>
<td>2</td>
<td>complete HS</td>
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<td>DQ. 17</td>
<td>House income</td>
<td>1</td>
<td>&lt; 20,000</td>
<td>2</td>
<td>21-30,000</td>
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<td>DQ. 18</td>
<td># of people in house</td>
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<td>DQ. 19</td>
<td>Grocery from?</td>
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<tr>
<td>DQ. 20</td>
<td>Rice?</td>
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<td>DQ. 21</td>
<td>Flour?</td>
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<td>TEL. REQ. RECEIVED BY:</td>
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<td>MSP</td>
<td>WCB</td>
<td>ICBC</td>
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<tr>
<th>Patient's Last Name</th>
<th>Doe</th>
<th>First Name</th>
<th>John</th>
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<tr>
<th>Address</th>
<th>City</th>
<th>Sex</th>
<th>D.O.B. (Y/M/D)</th>
<th>Age</th>
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<tr>
<th>Ordering Physician</th>
<th>Dr. R. Levy-Milne</th>
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<th>Ordering Physician's Address</th>
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<tr>
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<th>Practitioner No.</th>
<th>Date/Time of Medication:</th>
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</table>

### Chemistry

- Haemoglobin
- HGB only
- WBC
- WBC only
- Haematology Panel
- INR
- Glucose (Fasting) - see reverse for instructions
- Gestational Screen 50 gm load + 1 Hr. pc glucose
- Gestational Glucose Tolerance (100 gm) (3 Hr. test)
- Standard Glucose Tolerance (75 gm) (2 Hr. test)
- Pregnancy Test (Urinalysis)

### Microbiology

Label all Microbiology specimens.
Specify site of specimen -
- Nose
- Cervical
- Rectal
- Sputum
- Urethra
- Stool
- Throat
- Other
- Vaginal
  * See protocol below for urine culture

### MSP Protocols/Guidelines

Tests in the following section must be ordered in compliance with the protocol or guideline. Please provide diagnosis. *

- ESR * Diagnosis required - state below
- TSH * Diagnosis required - state below
- Serum Ferritin * Special case (List additional tests below)
- PSA Not for Screening (MSP billable)
- PSA Screening (Non-MSP billable)

### Lipids (Fasting) - see reverse for instructions / risk factors

- Risk Factor(s)/CAD/Medically indicated
  - Total Cholesterol
  - HDL Cholesterol
  - Triglycerides
  - LDL Cholesterol (calculated)

- Non-MSP billable
  - Total Cholesterol
  - HDL Cholesterol
  - Triglycerides
  - LDL Cholesterol (calculated)

### Hepatitis Testing & Reflex Testing For:

- Acute
- B Carrier
- Previous/Chronic

### Stool Ova & Parasites

- Single Specimen *
- High Risk (Times )

### Urine Testing

- Macroscopic (dipstick)
- Microscopic
- Macroscopic/Microscopic if indicated
- Macroscopic and Microscopic - * Special Case - Diagnosis required - state below
- Macroscopic/Culture if Pyuria or Nitrite Present
- Urine Culture

### Other Tests

- RCF, UHM

### Diagnosis

(please print in block capital letters)

**Date/Referring Physician Signature**

This requisition form, when completed for tests performed by BC Biomedical Laboratories Ltd., is a medical referral to Dr. C.J. Coady Associates

Owned & Operated by Dr. C.J. Coady Associates
Folate

Assay Summary

Sample Type: Serum, Red Blood Cells
Sample Volume: 150 μL
Calibrator: Folate
Sensitivity and Assay Range: 0.35 - 20 ng/mL (0.79 - 45.4 nmol/L)

Contents

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<thead>
<tr>
<th>Catalog Number</th>
<th>Contents</th>
<th>Number of Tests</th>
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<tr>
<td>110750</td>
<td>5 ReadyPack® primary reagent packs containing ADVIA® Centaur® Folate Lite Reagent, Solid Phase, and Releasing Agent ADVIA Centaur Folate Master Curve card</td>
<td>500</td>
</tr>
<tr>
<td>or</td>
<td>1 ReadyPack primary reagent pack containing ADVIA Centaur Folate Lite Reagent, Solid Phase, and Releasing Agent ADVIA Centaur Folate Master Curve card</td>
<td>100</td>
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Intended Use

For in vitro diagnostic use in the quantitative determination of folate in serum or red blood cells using the ADVIA® Centaur™ System.

Materials Required But Not Provided

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<th>Contents</th>
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<tr>
<td>672176</td>
<td>Folate Calibrator</td>
<td>6 vials of low calibrator 6 vials of high calibrator</td>
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<td>or</td>
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<tr>
<td>672175</td>
<td>Folate Calibrator</td>
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<td>110327</td>
<td>ADVIA Centaur Folate DTT</td>
<td>6 ReadyPack ancillary reagent packs containing 10 mL/pack</td>
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<td>or</td>
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<tr>
<td>110326</td>
<td>ADVIA Centaur Folate DTT</td>
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Optional Reagents

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<td>110315</td>
<td>ADVIA Centaur Multi-Diluent 3</td>
<td>2 ReadyPack ancillary reagent packs containing 5 mL/pack</td>
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<td>672192</td>
<td>Multi-Diluent 3</td>
<td>50 mL/vial</td>
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<td>672219</td>
<td>Folate Ascorbic Acid/Ascorbic Acid Diluent</td>
<td>25 mL/vial</td>
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<td>986000</td>
<td>Ligand Plus 1, 2, 3 quality control material</td>
<td>5 x 5 mL/level</td>
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<td>986400</td>
<td>Ligand Plus 1, 2, 3 barcode labels</td>
<td>60/level</td>
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<tr>
<td>672413</td>
<td>Folate Master Curve Material</td>
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Summary and Explanation of the Test

Folates are compounds of pteroylglutamic acid (PGA) that function as coenzymes in metabolic reactions involving the transfer of single-carbon units from a donor to a recipient compound. Folate, with vitamin B₁₂, is essential for DNA synthesis, which is required for normal red blood cell maturation.¹ Humans obtain folate from dietary sources including fruits,² green and leafy vegetables, yeast, and organ meats.³ Folate is absorbed through the small intestine and stored in the liver.

Low folate intake, malabsorption as a result of gastrointestinal diseases, pregnancy, and drugs such as phenytoin are causes of folate deficiency.⁴ Folate deficiency is also associated with chronic alcoholism.⁵ Folate and vitamin B₁₂ deficiency impair DNA synthesis, causing macrocytic anemias. These anemias are characterized by abnormal maturation of red blood cell precursors in the bone marrow, the presence of megaloblasts, and decreased red blood cell survival.⁶ Since both folate and vitamin B₁₂ deficiency can cause macrocytic anemia, appropriate treatment depends on the differential diagnosis of the deficiency. Serum folate measurement provides an early index of folate status.² However, folate is much more concentrated in red blood cells than in serum so the red blood cell folate measurement more closely reflects tissue stores.⁴ Red blood cell folate concentration is considered the most reliable indicator of folate status.²

Assay Principle

The ADVIA Centaur Folate assay is a competitive immunoassay using direct chemiluminescent technology. Folate in the patient sample competes with acridinium ester-labeled folate in the Lite Reagent for a limited amount of folate binding protein, which is covalently coupled to paramagnetic particles in the Solid Phase. The ADVIA Centaur Folate assay uses Releasing Agent (sodium hydroxide) and DTT to release the folate from endogenous binding proteins in the sample.

The system automatically performs the following steps:

• dispenses 150 μL of sample into a cuvette
• dispenses 50 μL of DTT
• dispenses 75 μL of Releasing Agent and incubates for 2.5 minutes at 37°C
• dispenses 200 μL of Solid Phase and incubates for 2.5 minutes at 37°C
• dispenses 100 μL of Lite Reagent and incubates for 2.5 minutes at 37°C
• separates, aspirates, and washes the cuvettes with reagent water⁶
• dispenses 300 μL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction
• reports results according to the selected option, as described in the system operating instructions or in the online help system

An inverse relationship exists between the amount of folate present in the patient sample and the amount of relative light units (RLUs) detected by the system.

Specimen Collection and Handling

Serum is the recommended sample type for this assay. Whole blood (heparin or EDTA) is required to measure red cell folate using the ADVIA Centaur Folate assay. The following recommendations for handling and storing blood samples are furnished by the National Committee for Clinical Laboratory Standards (NCCLS):⁷

• Collect all blood samples observing universal precautions for venipuncture.
• Allow samples to clot adequately before centrifugation.
• Keep tubes stoppered and upright at all times.
• Store samples at room temperature for no longer than 8 hours.
• Tightly cap and refrigerate specimens at 2 to 8°C if the assay is not completed within 8 hours.
• Freeze samples at or below -20°C if the assay is not completed within 48 hours.
• Freeze samples only once and mix thoroughly after thawing.

Before placing samples on the system ensure that:
• Samples are free of fibrin or other particulate matter.
• Samples are free of bubbles.

If testing is not done within 3 hours for whole blood specimens, determine the hematocrit and freeze the whole blood specimen or hemolysate. Frozen whole blood specimens can be stored at -20°C in a non-frost free freezer for up to 2 months. Bayer Diagnostics recommends preparing a hemolysate with the reconstituted Folate Ascorbic Acid before freezing. Samples diluted with Folate Ascorbic Acid can be stored at -20°C in a non-frost free freezer for up to 3 months. Freeze specimens only once and mix thoroughly after thawing.

For red blood cell folate determinations, prepare the red blood cell hemolysate as described in Preparing the Red Blood Cell Hemolysate.

**Preparing the Red Blood Cell Hemolysate**

1. Reconstitute the Folate Ascorbic Acid as described in *Reconstituting the Folate Ascorbic Acid*.
2. Collect the sample in a glass tube containing heparin or EDTA.
3. Invert the sample several times to mix.
4. Determine and record the hematocrit.
5. Dispense 1 mL of reconstituted Folate Ascorbic Acid into a test tube.
6. Add 50 µL of the sample into the Folate Ascorbic Acid.
7. Cap and invert the test tube several times to mix; do not vortex. Avoid foaming.
   **NOTE:** Protect the hemolysate from light.
8. Let the hemolysate stand at room temperature (18 to 25°C) for a minimum of 30 minutes. Do not exceed 120 minutes.
   **NOTE:** Freeze the hemolysate at or below -20°C immediately if testing cannot be completed within 3 hours from the time you begin preparing the hemolysate. Frozen whole blood specimens can be stored at or below -20°C in a non-frost free freezer for up to 2 months. Bayer Diagnostics recommends preparing a hemolysate with the reconstituted Folate Ascorbic Acid before freezing. Samples diluted with Folate Ascorbic Acid can be stored at or below -20°C in a non-frost free freezer for up to 3 months.

If the hemolysate is frozen, thaw the hemolysate and mix it by inverting the tube several times. Test the hemolysate within 3 hours after thawing.

For information about running the ADVIA Centaur Folate assay on the system, refer to Assay Procedure.
Store the reagents upright at 2–8°C. Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, Handling Reagents.

<table>
<thead>
<tr>
<th>Reagent Pack</th>
<th>Reagent</th>
<th>Volume</th>
<th>Ingredients</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADVIA Centaur Folate</td>
<td>Lite Reagent</td>
<td>10.0 mL/reagent pack</td>
<td>folate labeled with acridinium ester (−3 ng/mL) in buffer with sodium azide (0.13%) and preservatives</td>
<td>2–8°C</td>
<td>until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.</td>
</tr>
<tr>
<td>ReadyPack primary reagent pack</td>
<td>Solid Phase</td>
<td>20.0 mL/reagent pack</td>
<td>purified folate binding protein (bovine) (−0.002 mg/mL) covalently coupled to paramagnetic particles in buffer with sodium azide (0.14%) and preservatives</td>
<td>2–8°C</td>
<td>until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.</td>
</tr>
<tr>
<td></td>
<td>Releasing Agent</td>
<td>7.5 mL/reagent pack</td>
<td>sodium hydroxide (0.55N)</td>
<td>2–8°C</td>
<td>until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.</td>
</tr>
<tr>
<td>ADVIA Centaur Folate DTT</td>
<td>DTT</td>
<td>10.0 mL/reagent pack</td>
<td>dithiothreitol (29.4 mg/mL) in liquid form</td>
<td>2–8°C</td>
<td>until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.</td>
</tr>
<tr>
<td>ReadyPack ancillary reagent pack</td>
<td>Multi-Diluent 3</td>
<td>5.0 mL/reagent pack</td>
<td>human plasma with sodium azide (0.1%)</td>
<td>2–8°C</td>
<td>until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.</td>
</tr>
<tr>
<td></td>
<td>Folate Ascorbic Acid/ Ascorbic Acid Diluent</td>
<td>25.0 mL/vial</td>
<td>lyophilized ascorbic acid (0.025 g/vial)/folate-free human serum albumin (HSA) with sodium azide (&lt; 0.1%)</td>
<td>2–8°C</td>
<td>until the expiration date on the vial label. For onboard stability, refer to Onboard Stability and Calibration Interval.</td>
</tr>
</tbody>
</table>

**WARNING:** Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements. 

**Corrosive!** Causes burns. **Warning!** In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). **Contains:** sodium hydroxide; Releasing Agent

**Harmful!** Harmful if swallowed. **Warning!** After contact with skin, wash immediately with plenty of soap and water. **Contains:** sodium azide; Lite Reagent, Solid Phase

**POTENTIAL BIOHAZARD:** Human and/or other biological source material. Handle as if potentially infectious.

This product may contain one or more of the following materials:
- human serum or plasma, or other human source material
- biological source material of non-human origin
While each human serum or plasma donor unit used in the manufacture of this product was tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2 by FDA-approved methods, all products manufactured using human or non-human source material should be handled as potentially infectious. There are no approved tests for other human and/or non-human source material. Handle this product according to established good laboratory practices.

**Reconstituting the Folate Ascorbic Acid**

1. Add the entire contents of the Folate Ascorbic Acid Diluent to the lyophilized Folate Ascorbic Acid.
2. Let the reconstituted mixture stand at room temperature for 5 minutes and mix by inverting the bottle occasionally.

**Loading Reagents**

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

**CAUTION:** Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to Appendix C, *Handling Reagents*.

Load the ReadyPack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the Folate DTT ReadyPack reagent pack in the ancillary reagent entry. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

If automatic dilution of a sample is required, load ADVIA Centaur Multi-Diluent 3 in the ancillary reagent entry.

**Onboard Stability and Calibration Interval**

<table>
<thead>
<tr>
<th>Onboard Stability</th>
<th>Calibration Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 days</td>
<td>2 days</td>
</tr>
</tbody>
</table>

Additionally, the ADVIA Centaur Folate assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

**CAUTION:**

- Discard the primary reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

**Master Curve Calibration**

The ADVIA Centaur Folate assay requires a Master Curve calibration when using a new lot number of Lite Reagent and Solid Phase. For each new lot number of Lite Reagent and Solid Phase, use the barcode reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.
Defining Ratio and Off-System Tests for Red Blood Cell Folate (RBC Folate)

For more information about entering the off-system and ratio test definitions, refer to the system operating instructions or to the online help system.

You may define any name for the off-system test. Ensure that you use the same name in the ratio definition.

1. Enter the off-system test name for hematocrit, for example, HCT or HCRIT.
2. Save the changes.
3. Enter the ratio test name, for example, rbcFOL.
4. Enter the ratio test definition, for example, FOLATE x 2100/HCT using the following variables:
   a = Tests, FOL
   b = Variable, 0
   c = Tests, HCT
   d = Variable, 2100
5. Save the changes.

Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, two levels of quality control material should be assayed on each day that samples are analyzed. Quality control samples should also be assayed when performing a two-point calibration. Treat all quality control samples the same as patient samples.

For quality control of the ADVIA Centaur Folate assay, use Ligand Plus or an equivalent quality control material. Refer to the quality control product insert for the suggested Expected Values. If the quality control results do not fall within the suggested Expected Values or within the laboratory’s established values, then do the following:

- review these instructions to ensure that the assay was performed according to the procedures recommended by Bayer Diagnostics
- verify that the materials are not expired
- verify that required maintenance was performed
- if necessary, rerun the quality control samples or contact Bayer Diagnostics for more assistance

Sample Volume

This assay requires 150 μL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to Sample Volume Requirements in the ADVIA Centaur Reference Manual.
Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

**CAUTION:** Do not load more than one size of sample container in each rack. The rack indicator must be positioned at the correct setting for the size of sample container.

1. Prepare the sample container for each sample, and place barcode labels on the sample containers, as required.
2. Load each sample container into a rack, ensuring that the barcode labels are clearly visible through the slot in the rack.
3. Place the racks in the entry queue.
4. Start the entry queue, if required.

Procedural Notes

**Calculations**

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

The system reports serum folate results in ng/mL (mass units) or nmol/L (SI units), depending on the units defined when setting up the assay. The conversion formula is

\[ 1 \text{ ng/mL} = 2.27 \text{ nmol/L}. \]

**Red Blood Cell Folate**

Use this procedure to manually calculate RBC folate values, or program the system to calculate RBC folate values as described in the system operating instructions or in the online help system. Refer to *Defining Ratio and Off-System Tests for Red Blood Cell Folate (RBC Folate)*.

1. Multiply the folate result for the hemolysate by 21 (a 1:21 dilution was made when preparing the RBC hemolysate). This value represents the folate concentration of whole blood in ng/mL.

2. Divide this result by the hematocrit, and multiply by 100 to adjust for the hematocrit, which is a percentage.

\[ \text{RBC folate (ng/mL)} = \frac{(\text{Folate result for hemolysate, ng/mL}) \times 21 \times 100}{\text{hematocrit}} \]

**Example:**

- Hemolysate folate value = 5.7 ng/mL
- Hematocrit = 43
- \[ \text{RBC folate (ng/mL)} = \frac{5.7 \times 21 \times 100}{43} = 278 \]
Corrected Red Blood Cell Folate

In most cases the serum folate values are very small compared to red blood cell folate values. However, occasionally serum folate values can be elevated. If the serum folate value is high and the red blood cell folate concentration is low, calculate the corrected RBC folate value according to the following equation:

\[
\text{Corrected RBC folate (ng/mL)} = \frac{\text{RBC folate (ng/mL)} - \text{serum folate (ng/mL)}}{(100 \text{ hematocrit})} \]

Example:

- RBC folate = 210 ng/mL
- Serum folate = 22 ng/mL
- Hematocrit of the patient = 41

\[
\text{Corrected RBC folate (ng/mL)} = 210 - \frac{22}{100 - 41} = 210 - 32 = 178
\]

Dilutions

- Serum samples with folate levels greater than 20 ng/mL (45.4 nmol/L) must be diluted and retested to obtain accurate results.
- Patient samples can be automatically diluted by the system or prepared manually.
- For automatic dilutions, ensure that ADVIA Centaur Multi-Diluent 3 is loaded and set the system parameters as follows:
  - Dilution point: ≤ 20 ng/mL (45.4 nmol/L)
  - Dilution factor: 2

For detailed information about automatic dilutions, refer to the system operating instructions or to the online help system.

- Manually dilute the patient samples when patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.
- Use Multi-Diluent 3 to manually dilute patient samples, and then load the diluted sample in the sample rack, replacing the undiluted sample.
- Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner, and in compliance with all federal, state, and local requirements.

Limitations

Hemolysis significantly increases folate values due to the high folate concentrations in red blood cells. Methotrexate and leucovorin interfere with folate measurement because these drugs cross-react with folate binding proteins.

<table>
<thead>
<tr>
<th>Serum specimens that are...</th>
<th>Have an insignificant effect on the assay up to...</th>
</tr>
</thead>
<tbody>
<tr>
<td>lipemic</td>
<td>1000 mg/dL of triglycerides</td>
</tr>
<tr>
<td>icteric</td>
<td>20 mg/dL of bilirubin</td>
</tr>
</tbody>
</table>

Interference testing was determined according to NCCLS Document EP7-P.
Expected Results

The expected results for the ACS:180® Folate assay were previously established. To determine the reference range for serum and RBC folate, data was obtained on 617 and 827 samples, respectively.

<table>
<thead>
<tr>
<th>Category</th>
<th>Median Range</th>
<th>Median Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (ng/mL)</td>
<td>(nmol/L)</td>
</tr>
<tr>
<td>Serum folate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deficient*</td>
<td>57</td>
<td>1.6</td>
</tr>
<tr>
<td>normal</td>
<td>560</td>
<td>7.2</td>
</tr>
<tr>
<td>RBC folate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>deficient*</td>
<td>52</td>
<td>80.1</td>
</tr>
<tr>
<td>normal</td>
<td>775</td>
<td>295</td>
</tr>
</tbody>
</table>

* Diagnosed by bone and/or peripheral blood smear pathology and other criteria including:
  - megaloblastic anemia
  - folate deficient diet
  - malabsorption
  - alcoholism
  - Tropical Sprue
  - abnormal blood parameters including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and hematocrit (HCT).

For serum folate testing, based on the observed population, at 2.6 ng/mL (5.9 nmol/L), the overall diagnostic efficiency is 95%.

These results were confirmed for the ADVIA Centaur Folate assay by analyzing 308 serum samples in the range of 0.63 to 19.15 ng/mL (1.43 to 43.46 nmol/L). Refer to Method Comparison.

For RBC folate testing, based on the observed population, at 130 ng/mL (295 nmol/L), the overall diagnostic efficiency is 95%.

These results were confirmed for the ADVIA Centaur Folate assay by analyzing 277 red blood cell samples in the range of 16.19 to 1158.60 ng/mL (36.75 to 2630.02 nmol/L). Refer to Method Comparison.

As with all diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient results.

Performance Characteristics

Sensitivity and Assay Range

The ADVIA Centaur Folate assay measures folate concentrations up to 20 ng/mL (45.4 nmol/L) with a minimum detectable concentration (analytical sensitivity) of 0.35 ng/mL (0.79 nmol/L). Analytical sensitivity is defined as the concentration of folate that corresponds to the RLUs that are two standard deviations less than the mean RLUs of 20 replicate determinations of the Folate zero standard.

Method Comparison

For 308 serum samples in the range of 0.63 to 19.15 ng/mL (1.43 to 43.46 nmol/L), the relationship between the ADVIA Centaur Folate assay and the ACS:180 Folate assay is described by the equation:

ADVIA Centaur Folate = 0.98 (ACS:180 Folate) + 0.18 ng/mL

Correlation coefficient (r) = 0.98
For 277 red blood cell samples in the range of 16.19 to 1158.60 ng/mL (36.75 to 2630.02 nmol/L), the relationship between the ADVIA Centaur Folate assay and the ACS:180 Folate assay is described by the equation:

ADVIA Centaur RBC Folate = 0.96 (ACS: 180 Folate) + 6.20 ng/mL

Correlation coefficient (r) = 0.99

The RBC folate values from 12 individuals for samples collected with EDTA or with heparin were compared. Each mean was calculated from five values. The results are presented in the following table:

<table>
<thead>
<tr>
<th>(ng/mL)</th>
<th>Mean RBC Folate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>EDTA</td>
</tr>
<tr>
<td></td>
<td>(ng/mL)</td>
</tr>
<tr>
<td>391.1</td>
<td>887.7</td>
</tr>
<tr>
<td>333.7</td>
<td>757.4</td>
</tr>
<tr>
<td>456.4</td>
<td>1036.0</td>
</tr>
<tr>
<td>270.2</td>
<td>613.4</td>
</tr>
<tr>
<td>384.1</td>
<td>871.8</td>
</tr>
<tr>
<td>409.3</td>
<td>929.0</td>
</tr>
<tr>
<td>244.5</td>
<td>555.1</td>
</tr>
<tr>
<td>278.1</td>
<td>631.4</td>
</tr>
<tr>
<td>467.1</td>
<td>1060.4</td>
</tr>
<tr>
<td>476.9</td>
<td>1082.6</td>
</tr>
<tr>
<td>459.2</td>
<td>1042.4</td>
</tr>
<tr>
<td>611.3</td>
<td>1387.7</td>
</tr>
</tbody>
</table>

**Dilution Recovery**

Six human serum samples in the range of 9.44 to 14.94 ng/mL (21.43 to 33.91 nmol/L) of folate were diluted 1:2 and 1:4 with Multi-Diluent 3 and assayed for recovery and parallelism. The recoveries ranged from 100.6% to 117.3% with a mean of 107.4%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (ng/mL)</th>
<th>Expected (ng/mL)</th>
<th>Observed (nmol/L)</th>
<th>Expected (nmol/L)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>—</td>
<td>11.16</td>
<td>25.33</td>
<td>11.16</td>
<td>25.33</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>5.74</td>
<td>5.58</td>
<td>13.03</td>
<td>12.67</td>
<td>103.4</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>3.16</td>
<td>2.79</td>
<td>7.17</td>
<td>6.33</td>
<td>102.9</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>107.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td>14.94</td>
<td>33.91</td>
<td>14.94</td>
<td>33.91</td>
<td>106.7</td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>7.72</td>
<td>7.47</td>
<td>17.52</td>
<td>16.96</td>
<td>104.2</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>3.76</td>
<td>3.74</td>
<td>8.54</td>
<td>8.49</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>107.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>—</td>
<td>9.44</td>
<td></td>
<td>21.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>5.03</td>
<td>4.72</td>
<td>11.42</td>
<td>10.71</td>
<td>106.7</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>2.77</td>
<td>2.36</td>
<td>6.29</td>
<td>5.36</td>
<td>106.7</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>112.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td>12.98</td>
<td></td>
<td>29.46</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>6.76</td>
<td>6.49</td>
<td>15.35</td>
<td>14.73</td>
<td>104.2</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>3.54</td>
<td>3.25</td>
<td>8.04</td>
<td>7.38</td>
<td>106.6</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>107.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td>11.07</td>
<td></td>
<td>25.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>5.70</td>
<td>5.54</td>
<td>12.94</td>
<td>12.58</td>
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</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>108.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>—</td>
<td>10.44</td>
<td></td>
<td>23.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:2</td>
<td>5.60</td>
<td>5.22</td>
<td>12.71</td>
<td>11.85</td>
<td>107.4</td>
</tr>
<tr>
<td></td>
<td>1:4</td>
<td>2.81</td>
<td>2.61</td>
<td>6.38</td>
<td>5.92</td>
<td>107.6</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td></td>
<td>107.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Mean**

107.4
Spiking Recovery

Varying amounts of N-5-methyltetrahydrofolic acid were added to five samples with endogenous folate levels of 3.58 to 6.98 ng/mL (8.13 to 15.84 nmol/L). The recoveries ranged from 85.9% to 112.4% with a mean of 100.1%.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Endogenous Folate (ng/mL)</th>
<th>Amount Added (ng/mL)</th>
<th>Observed Folate (ng/mL)</th>
<th>Endogenous Folate (nmol/L)</th>
<th>Amount Added (nmol/L)</th>
<th>Observed (nmol/L)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.64</td>
<td>2.55</td>
<td>8.96</td>
<td>15.07</td>
<td>5.79</td>
<td>20.34</td>
<td>91.0</td>
</tr>
<tr>
<td></td>
<td>6.60</td>
<td>5.10</td>
<td>11.94</td>
<td>14.98</td>
<td>11.58</td>
<td>27.10</td>
<td>104.7</td>
</tr>
<tr>
<td></td>
<td>6.98</td>
<td>10.20</td>
<td>18.19</td>
<td>15.84</td>
<td>23.15</td>
<td>41.29</td>
<td>109.9</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>101.9</td>
</tr>
<tr>
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</table>

Precision

Four serum samples were assayed 6 times in 21 runs, on 5 systems (n = 126 for each sample), over a period of 6 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (ng/mL)</th>
<th>Mean (nmol/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.41</td>
<td>5.47</td>
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<td>8.1</td>
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<td>5.94</td>
<td>13.48</td>
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<td>3.6</td>
<td>5.3</td>
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<tr>
<td>12.35</td>
<td>28.03</td>
<td>3.7</td>
<td>4.0</td>
<td>5.5</td>
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<td>17.23</td>
<td>39.11</td>
<td>3.6</td>
<td>3.5</td>
<td>5.0</td>
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</tbody>
</table>

Three red blood cell samples were assayed 6 times in 17 runs, on 6 systems (n = 102 for each sample), over a period of 9 days. The following results were obtained:

<table>
<thead>
<tr>
<th>Mean (ng/mL)</th>
<th>Mean (nmol/L)</th>
<th>Within-run % CV</th>
<th>Run-to-run % CV</th>
<th>Total % CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>71.87</td>
<td>163.14</td>
<td>11.5</td>
<td>15.5</td>
<td>19.3</td>
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<tr>
<td>296.33</td>
<td>672.67</td>
<td>5.5</td>
<td>7.7</td>
<td>9.4</td>
</tr>
<tr>
<td>566.53</td>
<td>1286.02</td>
<td>4.4</td>
<td>6.6</td>
<td>7.9</td>
</tr>
</tbody>
</table>

Technical Assistance

For customer support, please contact your local technical support provider or distributor.
References

5. Lindenbaum J. Professor of Medicine, Columbia University, College of Physicians and Surgeons, New York, New York. Personal communication.

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US Pats 4,745,181; 4,918,192; 5,110,932; 5,241,070; 5,538,901; 5,609,822; 5,788,928
PLASMA HOMOCYSTEINE ASSAY

HOMOCYSTEINE
by Fluorescence Polarization on Abbott IMX

Principle

IMx Homocysteine is an enzyme immunoassay for the determination of total homocysteine in blood. Protein-bound homocysteine is reduced to free homocysteine and enzymatically converted to S-adenosyl-L-homocysteine (SAH) in a separate procedure prior to the immunoassay (FPIA).

Homocysteine (HCY) is a thiol-containing amino acid produced by the intracellular demethylation of methionine, an essential amino acid derived from dietary protein. Homocysteine is exported into plasma where it circulates, mostly in its oxidized form, bound to plasma proteins as a protein-HCY mixed disulfide with albumin. Smaller amounts of reduced homocysteine and the disulfide homocysteine (HCY-SS-HCY) are present. Total homocysteine (tHCY) represents the sum of all HCY species found in plasma or serum (free plus protein bound).

The IMx assay involves Reduction, Enzymatic conversion and Immunoassay. Plasma samples are mixed with buffer containing the reducing agent, dithiothreitol (DTT). Mixed disulfide and protein bound molecular forms of homocysteine are reduced to free homocysteine. As a result, all molecular forms are converted into one form, Homocysteine (total homocysteine). HCY is then enzymatically converted to S-adenosyl-L-homocysteine (SAH) by the use of SAH hydrolase and excess adenosine (Ad). SAH is then incubated with a monoclonal antibody (anti-SAH) and a fluorescently labeled tracer (S-adenosyl-cysteine). The amount of homocysteine can be estimated by Fluorescence Polarization Immunoassay (FPIA).

Summary of IMx assay:

<table>
<thead>
<tr>
<th>FREE HCY, HCY-PROT</th>
<th>DTT</th>
<th>HCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCY-S-S-HCY</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCY-S-S-CYSTEINE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reduction

<table>
<thead>
<tr>
<th>HCY + Excess Adenosine</th>
<th>SAH Hydrolase</th>
<th>SAH</th>
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</table>

Enzymatic Conversion

<table>
<thead>
<tr>
<th>Anti-SAH Antibody + Fluorecinated Tracer (S-adenosyl-cysteine)</th>
<th>Immunoassay (FPIA)</th>
<th>Quantitation of tHCY</th>
</tr>
</thead>
</table>

B.C. Biomedical Laboratories Ltd.
## OVERALL NUTRIENT INTAKES OF THE PARTICIPANTS

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basic Components</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calories</td>
<td>1373.67 ± 282.83</td>
<td>1408.02</td>
<td>595.79 – 1891.56</td>
</tr>
<tr>
<td>Calories from Fat</td>
<td>329.97 ± 103.14</td>
<td>322.7</td>
<td>139.63 – 593.47</td>
</tr>
<tr>
<td>Protein</td>
<td>52.73 ± 14.10</td>
<td>53.49</td>
<td>19.23 – 86.95</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>217.77 ± 45.15</td>
<td>219.94</td>
<td>99.46 – 332.83</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>23.04 ± 6.92</td>
<td>24.09</td>
<td>9.6 – 33.39</td>
</tr>
<tr>
<td>Soluble Fiber</td>
<td>3.44 ± 1.17</td>
<td>3.55</td>
<td>1.00 – 5.53</td>
</tr>
<tr>
<td>Sugar – Total</td>
<td>43.61 ± 20.16</td>
<td>40.03</td>
<td>5.98 – 98.58</td>
</tr>
<tr>
<td>Monosaccharides</td>
<td>8.78 ± 7.42</td>
<td>7.22</td>
<td>1.03 – 39.16</td>
</tr>
<tr>
<td>Disaccharides</td>
<td>6.75 ± 6.57</td>
<td>5.16</td>
<td>0.93 – 39.94</td>
</tr>
<tr>
<td>Other Carbohydrates</td>
<td>88.89 ± 28.33</td>
<td>87.44</td>
<td>42.88 – 155.12</td>
</tr>
<tr>
<td>Fat – Total</td>
<td>36.66 ± 11.46</td>
<td>35.86</td>
<td>15.51 – 65.94</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>11.17 ± 5.71</td>
<td>10.59</td>
<td>2.93 – 31.84</td>
</tr>
<tr>
<td>Mono Fat</td>
<td>12.75 ± 6.71</td>
<td>11.52</td>
<td>4.24 – 47.14</td>
</tr>
<tr>
<td>Poly Fat</td>
<td>5.73 ± 2.29</td>
<td>5.20</td>
<td>1.71 – 10.44</td>
</tr>
<tr>
<td>Trans Fatty Acids</td>
<td>0.57 ± 0.59</td>
<td>0.34</td>
<td>0.00 – 2.20</td>
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<tr>
<td>Cholesterol</td>
<td>92.44 ± 67.92</td>
<td>84.50</td>
<td>8.24 – 324.50</td>
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<tr>
<td><strong>Vitamins</strong></td>
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<tr>
<td>Vitamin A RE</td>
<td>534.96 ± 312.28</td>
<td>465.95</td>
<td>157.7 – 163.87</td>
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<tr>
<td>A – Carotenoid</td>
<td>276.56 ± 258.60</td>
<td>217.52</td>
<td>40.70 – 1353.10</td>
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<tr>
<td>A – Retinol</td>
<td>42.25 ± 36.31</td>
<td>34.73</td>
<td>4.09 – 190.78</td>
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<tr>
<td>A – Beta Carotene</td>
<td>1094.99 ± 1430.14</td>
<td>571.40</td>
<td>99.30 – 7732.00</td>
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<tr>
<td>Thiamin – B₁</td>
<td>1.53 ± 0.41</td>
<td>1.49</td>
<td>0.50 – 2.25</td>
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<tr>
<td>Riboflavin – B₂</td>
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<td>1.33</td>
<td>0.44 – 2.19</td>
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<td>Niacin – B₃</td>
<td>12.33 ± 3.57</td>
<td>12.50</td>
<td>3.22 – 20.36</td>
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<tr>
<td>Niacin Equivalents</td>
<td>19.78 ± 6.27</td>
<td>19.56</td>
<td>5.37 – 33.93</td>
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<tr>
<td>Vitamin – B₆</td>
<td>0.97 ± 0.37</td>
<td>0.94</td>
<td>0.26 – 2.4</td>
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<tr>
<td>Vitamin – B₁₂</td>
<td>1.70 ± 0.86</td>
<td>1.63</td>
<td>0.45 – 4.13</td>
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<tr>
<td>Vitamin C</td>
<td>91.98 ± 121.88</td>
<td>69.93</td>
<td>22.29 – 858.82</td>
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<tr>
<td>Vitamin D</td>
<td>1.95 ± 1.79</td>
<td>1.65</td>
<td>0.02 – 8.28</td>
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<tr>
<td>Vitamin E – Alpha Equivalents</td>
<td>2.85 ± 0.83</td>
<td>2.83</td>
<td>0.54 – 4.57</td>
</tr>
<tr>
<td>Folate</td>
<td>411.29 ± 103.72</td>
<td>389.26</td>
<td>197.90 – 639.01</td>
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<tr>
<td>Pantothenic Acid</td>
<td>2.96 ± 1.02</td>
<td>2.88</td>
<td>1.11 – 5.70</td>
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<td>Minerals</td>
<td>Value ± Standard Error</td>
<td>Minimum</td>
<td>Maximum</td>
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<tr>
<td>Calcium</td>
<td>65.48 ± 271.75</td>
<td>652.75</td>
<td>113.47 – 1341.01</td>
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<td>Copper</td>
<td>0.83 ± 0.25</td>
<td>0.81</td>
<td>0.36 – 1.72</td>
</tr>
<tr>
<td>Iron</td>
<td>15.91 ± 4.56</td>
<td>16.05</td>
<td>8.24 – 24.32</td>
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<tr>
<td>Magnesium</td>
<td>223.08 ± 78.71</td>
<td>211.70</td>
<td>100.16 – 479.09</td>
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<td>Manganese</td>
<td>3.22 ± 1.26</td>
<td>2.94</td>
<td>0.72 – 7.06</td>
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<tr>
<td>Phosphorus</td>
<td>1073.82 ± 296.02</td>
<td>1057.21</td>
<td>360.46 – 1617.98</td>
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<td>Potassium</td>
<td>2013.68 ± 577.43</td>
<td>2057.87</td>
<td>910.77 – 4106.22</td>
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<td>Selenium</td>
<td>36.79 ± 23.40</td>
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<td>5.62 – 92.51</td>
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<td>Sodium</td>
<td>115.48 ± 464.97</td>
<td>1119.74</td>
<td>116.21 – 2235.27</td>
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<td>Zinc</td>
<td>14.41 ± 60.19</td>
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<tr>
<th>Other Fats</th>
<th>Value ± Standard Error</th>
<th>Minimum</th>
<th>Maximum</th>
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<tbody>
<tr>
<td>Omega 3 Fatty Acids</td>
<td>0.67 ± 0.21</td>
<td>0.67</td>
<td>0.18 – 1.09</td>
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<td>Omega 6 Fatty Acids</td>
<td>3.02 ± 1.14</td>
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<td>0.60 – 6.69</td>
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<table>
<thead>
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<th>Other</th>
<th>Value ± Standard Error</th>
<th>Minimum</th>
<th>Maximum</th>
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</thead>
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<tr>
<td>Alcohol</td>
<td>0.10 ± 0.67</td>
<td>0.00</td>
<td>0.00 – 4.52</td>
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<tr>
<td>Caffeine</td>
<td>117.45 ± 64.17</td>
<td>117.6</td>
<td>0.00 – 302.74</td>
</tr>
</tbody>
</table>

*n = 45

NOTE: This study was not designed to assess the intakes of all of the above nutrient mentioned. The above table is presented only to be used as a crude check. Dietary data collection was done only keeping in mind the objective of assessing the folate intake of subjects.