A COMPARISON OF FOLIC ACID PHARMACOKINETICS IN OBESE AND NON-OBESE WOMEN OF CHILDBEARING AGE: IMPLICATIONS FOR PERICONCEPTIONAL FOLIC ACID DOSING

by

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ABSTRACT

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Obesity in pregnancy has been associated with an elevated risk for neural tube defects, though it is unknown if this is linked to a lower folate status in obese women. Studies have identified a reduced folate status among obese women even after controlling for folate intake. Thus, it is possible that folic acid pharmacokinetics are altered in the obese body. In this study, we compared the pharmacokinetics of folic acid in obese and non-obese women of childbearing age, following administration of a weight-adjusted dose. Area under the concentration-time curve was found to be significantly higher in the obese group, with the dose per kilogram lean body weight most strongly predicting systemic exposure. Estimation of the daily dose required to achieve protective blood concentrations did not identify a need to change supplementation recommendations for obese women. Accordingly, current guidelines appear to suggest adequate doses for obese women of childbearing age.

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To Mom, Dad, Maya, and Benji, thank you for your unconditional love and support. And to Beckie, thank you for standing by me every step of the way. You have all played a pivotal role in each of my accomplishments.

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LIST OF ABBREVIATIONS

AdoMet	*S*-adenosylmethionine
AICAR	aminoimidazole-4-carboxamide ribonucleotide
AUC	area under the concentration-time curve
BMI	body mass index
CDC	Centers for Disease Control and Prevention
CI	confidence interval
CL	total apparent clearance rate
C_{max}	maximum concentration
CNS	central nervous system
DHF	dihydrofolate
DFE	dietary folate equivalents
dTMP	deoxythymidine monophosphate
FR	folate receptor
GAR	glycinamide ribonucleotide
k_{el}	elimination rate constant
LBW	lean body weight
MTHFR	5,10-methylenetetrahydrofolate reductase
NHANES	National Health and Nutrition Examination Survey
NTD	neural tube defect
OR	odds ratio
PCFT	proton coupled folate transporter
RBC	red blood cell
RCT	randomized controlled trial

RFC	reduced folate carrier
RR	relative risk
SHMT	serine hydroxymethyltransferase
SOGC	Society of Obstetricians and Gynaecologists of Canada
$t_{1/2}$	half-life
TBW	total body weight
THF	tetrahydrofolate
t_{max}	time to maximum concentration
V_d	volume of distribution

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CHAPTER 1. INTRODUCTION

1.1. Statement of the Problem

Since the early 1990s, studies have established an association between obesity and an elevated risk for neural tube defects (NTD). As obesity is associated with various conditions that also constitute risk factors for NTDs, including folate deficiency, several studies have attempted to control for these confounders. Unfortunately, no variables were identified to contribute to the increased risk of NTDs among obese mothers. Even after adjusting for adequate folic acid supplementation during the periconceptional period, the elevated risk remains.

However, it is possible that an obese woman handle folic acid differently, thus requiring an adjusted dose to maintain a protective blood folate status. Several investigations have noted that, when controlling for absolute intake, obese individuals often have a lower folate status. Accordingly, a need exists to compare the pharmacokinetics of folic acid between obese and non-obese women of childbearing age. With obesity affecting such a large proportion of the population, understanding the relationship between obesity and folic acid pharmacokinetics will allow for a more targeted approach in the development of dosing guidelines.

1.2. Purpose and Study Objectives

The purpose of this study was to assess whether obese women of reproductive age handle folic acid differently from non-obese women of reproductive age. The following two objectives were employed in this investigation:

Objective 1: To compare folic acid pharmacokinetics in obese and non-obese women of childbearing age.

Objective 2: To estimate the daily folic acid dose required in achieving and maintaining steady-state blood folate concentrations that reduce the risk for NTDs for women across the spectrum of body weight.

1.3. Research Hypotheses and Rationale

The hypotheses matching each of the study objectives are as follows:

Hypothesis 1: Previous studies have found a lower folate status among obese individuals, even after controlling for absolute folate intake. We hypothesized that administration of equivalent doses of folic acid per kilogram total body weight will lead to similar levels of systemic folate exposure between obese and non-obese women of childbearing age.

Hypothesis 2: As the current literature indicates that the obese woman displays a reduced response to a given dose of folic acid, we hypothesize that obese women of childbearing age will require, on average, a larger daily dose in order to achieve and maintain steady-state blood folate concentrations that reduce the risk for NTDs.

CHAPTER 2. REVIEW OF THE LITERATURE

2.1. Neural Tube Defects

2.1.1. Description and characteristics

NTDs are a class of congenital malformations characterized by the failure of the neural tube to close properly during embryogenesis. The neural tube, which acts as a precursor to both the central nervous system (CNS) and much of the peripheral nervous system (Cabrera *et al*., 2004), is typically fully developed and fused by 21-28 days post-conception (Botto *et al*., 1999). A failure of several complex processes involved in the formation of the neural tube results in NTDs, most commonly occurring as either spina bifida (incomplete closure at the caudal end) or anencephaly (incomplete closure at the cranial end); less common classifications include craniorachischisis, encephalocele, and iniencephaly (Figure 1).

As the severity of the condition falls along a spectrum, infants born with NTDs display a wide range of disabilities. Those afflicted by anencephaly are unable to survive following birth, as vital brain regions are undeveloped and large sections of the remaining brain regions remain unprotected by the skull. However, various treatments, including surgical management, have allowed those born with spina bifida to survive. Recent advancements have enabled *in utero* surgical treatment, preventing potential complications arising during birth (Adzick *et al*., 1998). For these children, long-term prognoses can range from asymptomatic cases to those involving various degrees of physical disability and paralysis, reduced IQ, and impaired psychosocial development (Date *et al*., 1993). Survival

rates for those born with open spina bifida has been approximated at 79% after one year, 66% after five years, and 52% at 26 years (Hunt, 1997). Total lifetime costs of treatment for an individual with an NTD have been estimated at nearly $300,000, including medical costs and educational services (Botto *et al.*, 1999).

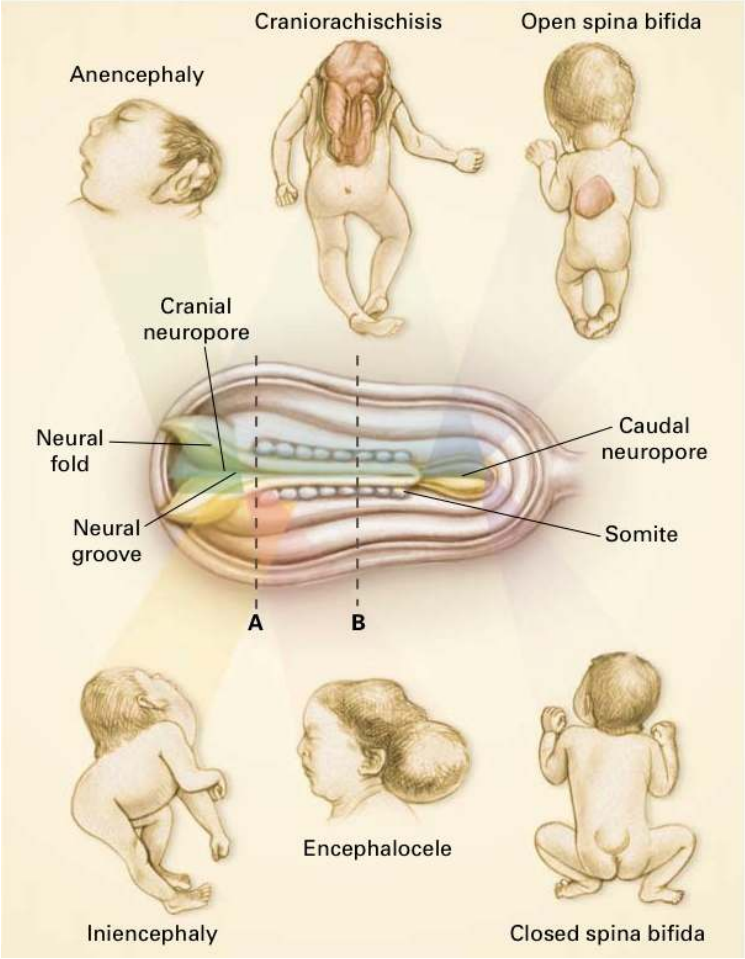

Figure 1. Anatomical features of the neural tube and various neural tube defects (Adapted from Botto *et al*., 1999)

The diagram depicts the dorsal view of the neural tube, closed in the center (around dotted line B), but open towards the cranial end (beyond dotted line A). If properly developed, the neural folds will seal over the neural groove, forming the neural tube. A failure of this process will result in a neural tube defect, depicted in the surrounding graphics. Reprinted from New England Journal of Medicine, Vol. 341 (20), Botto *et al*. Neural-tube defects. Pg 1509-19. Copyright 1999, with permission from the New England Journal of Medicine.

2.1.2. Prevalence and disease burden

In 1998, NTDs were estimated to affect nearly 300,000 pregnancies worldwide (Shibuya and Murray, 1998). Currently, NTDs are the second most common major malformation observed in live infants, with congenital heart defects being the most prevalent (Blom, 2009). Prevalence rates of NTDs approximate 1 in 1000 births in the developed world, though the incidence varies greatly according to ethnicity, geography, and folate exposure, with worldwide estimates ranging from 0.5 to 8 in 1000 births (Fleming, 2001). Following fortification of grain products with folic acid in Canada, prevalence rates fell from 1.58 in 1000 to 0.86 in 1000, with provincial discrepancies ranging from a low of 0.67 in 1000 in Alberta to a high of 1.26 in 1000 in Nova Scotia (De Wals *et al*., 2007). In the United States, post-fortification prevalence rates fell to an average of 0.54 in 1000 births, though notable ethnic discrepancies remain (Boulet *et al*., 2008). Hispanic mothers continue to have higher rates of NTD-affected pregnancies than either non-Hispanic white or non-Hispanic black mothers (Centers for Disease Control and Prevention, 2009).

Elsewhere in the world, NTD prevalence rates have been found to be substantially higher. The Shanxi Province of northern China, where fortification has not been implemented, is documented as having the highest worldwide incidence of NTDs, with nearly 14 in 1000 births affected in 2003 (Li *et al*., 2006). Ireland, where uptake of NTD prevention techniques is low and termination of pregnancy is illegal (McGuire *et al*., 2010), has consistently demonstrated the highest incidence of NTDs among European countries (EUROCAT Working Group, 1991). Yet, despite many years of experience with folate

fortification and recent debate on the issue (Sutton *et al*., 2008), fortification has yet to be approved and implemented in this region (Timotijevic *et al*., 2011).

2.1.3. Etiology and risk factors

Despite decades of reports and discussions of NTDs in the literature, little is understood regarding the mechanisms that precipitate these major malformations. Both environmental and genetic factors have been proposed as causative agents, though it is likely that most occurrences are an outcome of some combination of these risk factors (Dunlevy *et al*., 2007). Different mechanisms are believed to obstruct processes related to cellular proliferation, vascularization, DNA methylation, and proper shape formation of the neural tube during the period of neurulation (Cabrera *et al*, 2004). Many genes associated with NTDs have been linked to folate metabolism, and several animal studies have identified folate-resistant knockout models (Copp *et al*., 2003), though few candidate genes have been found to confer a strong association (Au *et al*., 2010). Appropriately, much research has been focused on the involvement of folate in the etiology of NTDs.

Since the 1960s, maternal folate deficiency has been realized as a considerable risk factor for NTD-affected births. In 1965, Hibbard and Smithells found an increased risk for CNS malformations in infants born to mothers with defective folate metabolism (Hibbard and Smithells, 1965). Smithells and colleagues subsequently hypothesized that the elevated risk for NTDs among infants born in the United Kingdom to mothers of lower social classes may be a consequence of differences in nutritional intake (Smithells *et al*., 1976). A comparison of blood vitamin status among mothers of various social classes found lower

values for red blood cell (RBC) folate, vitamin C, and riboflavin, concomitant with a higher incidence of NTDs. This study prompted investigations into the efficacy of vitamin supplementation prior to conception in the primary prevention of NTDs.

In addition to folate deficiency, several other NTD risk factors have been proposed. Exposure to environmental contaminants, such as organic solvents, heavy metals, and pesticides, has been linked to an increased incidence of NTDs (Sever, 1995). As well, maternal use of certain medications (e.g. valproic acid) (Lammer *et al*., 1987), diabetes (Becerra *et al*., 1990), hyperthermia (Edwards *et al*., 1995), certain genetic polymorphisms (Boyles *et al*., 2005), and obesity (Waller *et al*., 1994) have all been identified as NTD risk factors.

2.2. Folic Acid

2.2.1. Properties and sources

Folic acid, or pteroylmonoglutamic acid, refers to the oxidized form of folate, the water-soluble B-group vitamin. The term 'folate' was derived from the Latin word *'folium'*, or leaf, as folate was initially purified from spinach (Mitchell *et al*., 1941). Folates are naturally synthesized in abundance in green, leafy vegetables, various legumes, and in moderate amounts in certain fruit juices. Animal liver contains high concentrations of folate, as the liver is the primary storage site for the vitamin. As mammals are unable to endogenously synthesize folate, the vitamin must be consumed in order to maintain adequate physiological concentrations.

The folate molecule is composed of a pteroate substituent, including an aromatic pteridine ring attached to *p*-aminobenzoate, joined to a glutamic acid substituent (Quinlivan *et al*., 2006) (Figure 2). Most natural folates do not contain only a single glutamate moiety; rather, they exist in polyglutamate forms, linked by γ-glutamyl bonds (Lucock, 2000). These polyglutamates are more readily retained by intracellular proteins, thus allowing regulation of folate-enzyme activity (Quinlivan *et al*., 2006). Various folate species further differ in the one-carbon units located on nitrogens N5 and/or N10, each formed by the addition of moieties to the reduced folate species, tetrahydrofolate (THF). For instance, 5-methyl-THF, the most common species found in circulating plasma, is formed by the addition of a methyl group on N5. Other predominant forms include 5-formyl-THF, 10-formyl-THF and 5,10-methylene-THF.

Folic acid, which is fully oxidized and does not contain any one-carbon unit substituents, is not naturally synthesized in plants. Its oxidized structure allows for greater stability in various environments. Thus, it is less susceptible to degradation by storage and processing, and, consequently, is frequently used in supplement form and for fortifying food products.

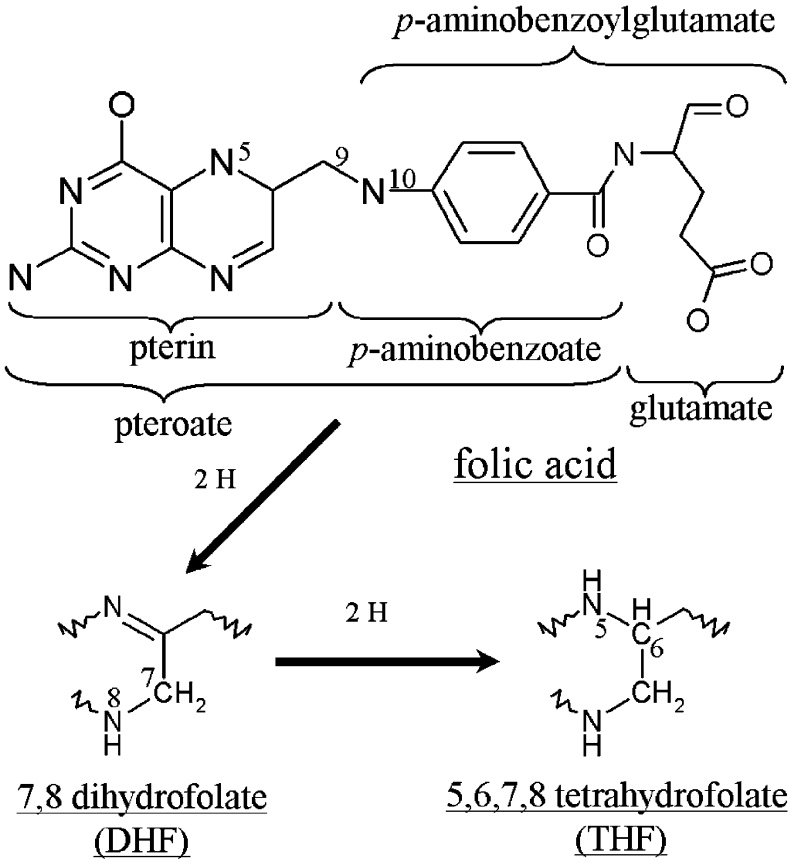

Figure 2. Molecular structures of folic acid, dihydrofolate, and tetrahydrofolate (Adapted from Quinlivan *et al.*, 2006)

Folic acid is composed of both pterin and *p*-aminobenzoate moieties (pteroate), linked to a single glutamate residue. Endogenously, folic acid is reduced first to dihydrofolate at C7 and N8, and then further reduced to tetrahydrofolate at N5 and C6. Reprinted from Analytical Biochemistry, Vol. 348 (2), Quinlivan *et al*. The analysis of folate and its metabolic precursors in biological samples. Pg 163-84. Copyright 2006, with permission from Elsevier.

2.2.2. Folate pharmacokinetics

2.2.2.1. Bioavailability

Folic acid bioavailability differs from that of natural folates, with synthetic folic acid often approaching nearly 100% bioavailability (Melse-Boonstra *et al*., 2004). Natural, dietary folates are estimated to be approximately 50% bioavailable (Sauberlich *et al*., 1987). When consumed as part of a fortified food product, rather than as a supplement, folic acid bioavailability decreases to around 85% (Pfeiffer *et al*., 1997). This difference in bioavailability is due to several factors, including the length of the polyglutamate chains, the stability of oxidized folate, and the obstruction of natural folate absorption by other dietary factors (e.g. fibre) (Gregory *et al*., 1992).

These variations have led to the formation of Dietary Folate Equivalents (DFE), used to equate all forms of folate intake to that of dietary folate (Bailey, 1998). Based on the absorption differences between fortified food and natural folates, folic acid consumed alongside a meal or as part of a fortified product was estimated to be 1.7-fold more bioavailable than dietary folate. Accordingly, naturally occurring folates are equivalent to 1 DFE per 1 µg folate, while synthetic folic acid is equivalent to 1.7 DFE per 1 µg folic acid.

2.2.2.2. Absorption

While folic acid is absorbed unchanged, natural folates, which rarely occur in the monoglutamate form, must first be hydrolyzed to remove the polyglutamate chains (Pietrzik *et al*., 2010). The enzyme for this, glutamate carboxypeptidase II, is present in the brush border of the small intestine (Lucock, 2000). Glutamate carboxypeptidase II removes

single glutamate residues until the folate exists as a monoglutamate, whereas it can then be transported across the brush border membrane in the proximal region of the small intestine (Said *et al*., 2000).

Both folic acid and reduced folates are absorbed across the intestinal membrane using the proton coupled folate transporter (PCFT) (Zhao *et al*., 2009). This transporter functions most efficiently in the acidic environment of the intestine, ideally around a pH of 6.0 (Selhub *et al*., 1987), and is responsible for a majority of folate absorption. The reduced folate carrier (RFC) is additionally responsible for folate absorption; however, its affinity for folic acid is substantially lower than that of reduced folates, and thus plays a minimal role in the absorption of supplemented folic acid.

2.2.2.3. Metabolism

Metabolism of folic acid and natural folates occurs prior to entering systemic circulation. During transport across the intestinal mucosa, dihydrofolate reductase (DHFR) reduces folates to dihydrofolate (DHF), and subsequently to THF (Pietrzik *et al*., 2010). THF is converted via serine hydroxymethyltransferase (SHMT) to 5,10-methylene-THF, and then to 5-methyl-THF via 5,10-methylenetetrahydrofolate reductase (MTHFR), though there is disagreement on whether this occurs first in the intestinal mucosa or the liver (Wright *et al*., 2007). 5-methyl-THF is released into systemic circulation as the predominant active folate species, and is most easily transported into peripheral tissues. At doses of greater than 200 µg of folic acid, oxidized folates have been detected unchanged in circulating plasma (Schmitz *et al*., 1994).

2.2.2.4. Distribution

After leaving the intestinal mucosa, folates are initially delivered to the liver, the primary site for folate storage in the body, where PCFT is responsible for uptake into hepatocytes (Zhao *et al*., 2009). In the liver, polyglutamate chains are added to much of the entering folates by folylpolyglutamate synthetase, thereby ensuring cellular retention for storage. 5-methyl-THF must be converted to THF by methionine synthase (MS) prior to polyglutamylation (Sirotnak and Tolner, 1999). Consequently, high endogenous concentrations of folate must compete for demethylation to THF, ensuring homeostatic maintenance of physiological tissue levels (Pietrzik *et al*., 2010).

Remaining folate stores are either released into the bile for elimination and/or reabsorption, or released into circulation for delivery to periphery tissues. Circulating folate is both free and loosely protein-bound (Pietrzik *et al*., 2010), and is transported into peripheral tissues via RFC or the folate receptor (FR) (Zhao *et al*., 2009).

2.2.2.5. Elimination

At low levels of folate intake, elimination largely occurs via biliary excretion (Pietrzik *et al*., 2010). Much of this is reabsorbed through enterohepatic recycling pathways. Folates are also filtered at the glomerulus, but are mostly reabsorbed at physiological levels via the FR (Goresky *et al*., 1963). Only at higher levels of folate intake, and higher serum concentrations, are significant amounts of folate lost to feces and urine. This has been shown to occur at serum levels exceeding 45 nmol/L (de Meer *et al*., 2005).

Catabolism of the folate molecule occurs via cleavage of the C9-N10 bond, forming pterine and *p*-amino-benzylpolyglutamate (Suh *et al*., 2001). While the pterine moiety is excreted unchanged in the feces, the *p*-amino-benzylpolyglutamate moiety is first hydrolyzed and acetylated prior to urinary excretion (Pietrzik *et al*., 2010). At supraphysiological levels, unchanged folates have been detected in both urine and feces (Suh *et al*., 2001).

Overall, whole-body turnover of folate stores has been approximated at over 100 days (Stites *et al*., 1997). RBC folate, which is incorporated into erythrocytes during erythropoiesis, exhibits a half-life of approximately 8 weeks (Pietrzik *et al*., 2007).

2.2.3. Folate bio-utilization

Folate plays a critical role in cell growth and replication owing to its actions as a participant in one-carbon transfer. Intracellularly, folate is involved in several major reactions, including the synthesis of thymidylate, purines, and methionine, as well as the catabolism of histidine and the conversion of serine to glycine (Lucock, 2000) (Figure 3). As methionine is directly involved in the methylation of DNA, proteins, and lipids, folate is additionally indirectly involved in regulating levels of DNA methylation. After entering the cell as 5-methyl-THF, folate is demethylated to THF, allowing it to participate in various one-carbon reactions.

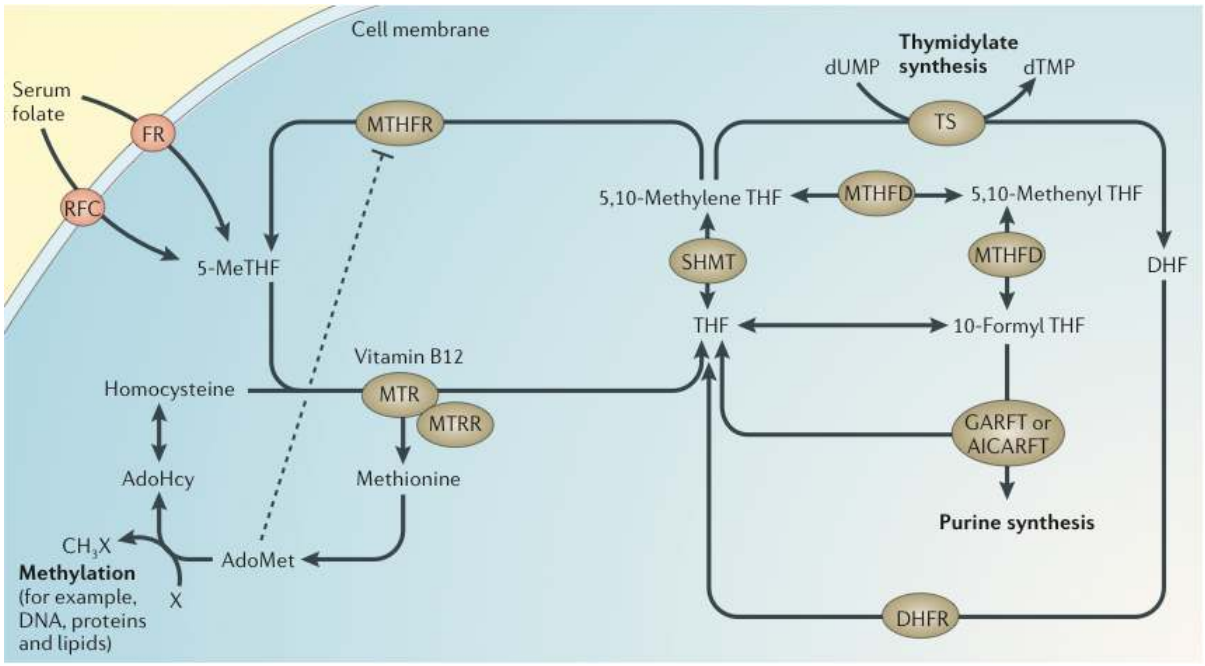

Figure 3. Major intracellular folate metabolic pathways (Adapted from Blom *et al*., 2006)

Folate is transported into cells via either FR or RFC. 5-MeTHF must first be demethylated to THF before involvement in purine or thymidylate synthesis pathways. Demethylation is mediated by MTR, which remethylates homocysteine to methionine. Methionine can participate in methylation of various DNA, proteins, and lipids. 5-MeTHF, 5-methyltetrahydrofolate; AdoHcy, S-adenosylhomocysteine; AdoMet, S-adenosylmethionine; AICARFT, aminoimidazolecarboxamine ribonucleotide transformylase; DHF, dihydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; FR, folate receptor; GARFT, glycinamide ribonucleotide transformylase; MTHFD, methylenetetrahydrofolate dehydrogenase; MTHFR, methylenetetrahydrofolate reductase; MTR, methionine synthase; MTRR, methionine synthase reductase; RFC, reduced folate carrier; SHMT, serine hydroxymethyltransferase; THF, tetrahydrofolate; TS, thymidylate synthase. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neuroscience; Blom et al. Neural tube defects and folate: case far from closed. 7(9), p724-731. Copyright 2006.

2.2.3.1. Purine and pyrimidine biosynthesis

In order to be directed towards purine synthesis, THF must first be converted to 10-formyl-THF using formate and ATP (Lucock, 2000). 10-formyl-THF then donates a carbon to both aminoimidazole-4-carboxamide ribonucleotide (AICAR) and glycinamide ribonucleotide (GAR), via AICAR transformylase and GAR transformylase, respectively. These reactions form the foundation of the purine ring.

In order to be directed towards pyrimidine synthesis, THF must be converted to 5,10-methylene-THF via SHMT. 5-10-methylene-THF is than able to methylate deoxyuridine monophosphate to deoxythymidine monophosphate (dTMP) via thymidylate synthase. This synthesis of dTMP is the rate-limiting step in the formation of DNA, and, thus, is targeted by anti-metabolites such as methotrexate (Lucock, 2000).

2.2.3.2. Serine and glycine interconversion

Alongside the conversion of THF to 5,10-methylene-THF, SHMT utilizes vitamin B_6 to demethylate serine (transferring the carbon to 5,10-methylene-THF) and form glycine (Stover and Schirch, 1993). As 5,10-methylene-THF can be directed towards the synthesis of methionine, purines, or thymidine, this step is highly regulated in mammalian cells (Lucock, 2000). When serine levels are high, serine can be converted to formate in the mitochondria, and additionally directed towards conversion of THF to 10-formyl-THF (Kastanos *et al*., 1997).

2.2.3.3. Methylation cycle

Once converted to 5-methyl-THF by MTHFR (or after entering the cell in this form), THF can only be recovered following the conversion of homocysteine to methionine (van der Put *et al*., 2001). This reaction is completed by methionine synthase, which utilizes vitamin B_{12}, or cobalamin, as a cofactor. Methionine can then be converted to *S*-adenosylmethionine (AdoMet) using single-carbon units from various amino acids. AdoMet acts as the methyl donor in the methylation of DNA, proteins, and lipids. At high concentrations, AdoMet reduces MTHFR activity, thus shunting intracellular folate activity

to purine and pyrimidine synthesis pathways (Blom *et al*., 2006). As folate is necessary for the remethylation of homocysteine to methionine, low folate intake has been associated with elevated circulating homocysteine concentrations (Rasmussen *et al*., 2000).

2.2.4. Quantification of blood folate status

As with most vitamins, measures of folate status are most frequently obtained from blood samples. Two different indices of folate status are typically used: serum folate and RBC folate. Both of these indicators have several benefits and drawbacks. However, these methods are preferred to the use of dietary intake as an index of folate status, as folate intake has proven less reliable when compared to measures of blood status (Weinstein *et al*., 2001).

Serum folate is preferred in the quantification of recent folate status, as it is more sensitive to short-term dietary intake (Drogan *et al*., 2004). Serum folate increases rapidly following consumption of folic acid or dietary folate, and excess folate is eliminated from the body over the course of several hours (Nguyen *et al*., 2009). Accordingly, blood tests obtained to measure background serum folate must be taken while fasting, in order to avoid inflated calculations.

RBC folate is preferred in the quantification of long-term folate status, as folate is incorporated into erythrocytes only during their formation in the bone marrow, and thus, RBC folate is insensitive to recent dietary intake (Drogan *et al*., 2004). Consequently, RBC folate measurements do not need to be obtained from fasting blood samples. As erythrocytes have a lifespan of approximately 120 days, RBC folate is reflective of folate

status for several months prior to obtaining the blood sample. Accordingly, experimentally induced folate deprivation will produce clinically deficient serum folate levels (<3 ng/mL) in about 3 weeks, while clinically deficient RBC folate levels (<20 ng/mL) do not become apparent until about 4 months (Herbert, 1967).

Despite these differences, several studies have found relatively strong correspondence between the two indices. An analysis of serum folate and RBC folate from 363 participants in the European Investigation into Cancer and Nutrition study found both measurements correlated reasonably well ($r = 0.63$, $p < 0.0001$) (Drogan *et al*., 2004). Similar findings were reached by an analysis of 1,259 patients by Phekoo and colleagues (Phekoo *et al*., 1997). These outcomes, along with the cost and increased difficulty of conducting RBC folate measurement assays, have led some groups to suggest that, with several exceptions, serum folate assays are sufficient in quantifying folate status when testing for folate deficiency (Galloway and Rushworth, 2003).

2.2.5. Folic acid and pregnancy

During pregnancy, maternal folate requirements increase between 5 and 10 times the amounts typically required for a non-pregnant woman, as folate becomes diverted towards the fetus and placenta, as well to supporting various maternal tissues (Antony, 1996). Both serum and RBC folate concentrations become markedly higher in the fetus and the placenta, as compared to maternal compartments (Giugliani *et al*., 1985). Correspondingly, low maternal folate status has been associated with several birth defects in fetal anatomical regions particularly sensitive to reduced folate uptake (Finnell *et al*.,

1998), including oral cleft, cardiovascular defects, and NTDs (Oyama *et al*., 2009). Unfortunately, little is known regarding the involvement of folate in these malformations, though proposed factors include reduced methylation, reduced DNA synthesis, and altered folate metabolism (Blom *et al*., 2006, Rothenberg *et al*., 2004).

2.2.5.1. History of folate and NTD prevention

Realizing that reduced folate status was associated with an increased risk for NTDs, Smithells and colleagues conducted the first randomized controlled trial (RCT) in 1980 to evaluate the efficacy of multivitamin supplementation in reducing the incidence of these birth defects (Smithells *et al*., 1980). Several hundred women planning pregnancy and with a history of an NTD-affected birth were recruited, and randomized to either a control or folic acid-containing multivitamin group. Of these participants, 4.0% of the control group gave birth to an NTD-affected infant, while only 0.5% of children born to the multivitamin group were affected by this malformation (Smithells *et al*., 1981).

However, as this trial used a folic acid-containing multivitamin, rather than folic acid alone, it was not yet possible to conclude that folic acid was reducing the risk for NTDs among these women. To evaluate the efficacy of folic acid in reducing NTD risk among women with a history of these birth defects, the landmark Medical Research Council Vitamin Study was conducted in the United Kingdom (MRC Vitamin Study Research Group, 1991). Women at risk of NTD-affected pregnancies (n = 1817) were randomized to one of four groups: folic acid (4 mg), a multivitamin not containing folic acid, folic acid plus the multivitamin, or no treatment. While the non-folic acid groups did not demonstrate any

reduced risk for NTDs, those receiving folic acid demonstrated a reduced incidence of NTDs by 72% (RR 0.28, 95% CI 0.12-0.71).

By 1994, a subsequent RCT was completed to evaluate the efficacy of folic acid supplementation in reducing the risk of NTDs among first-time mothers (Czeizel and Dudás, 1992). A total of 5,502 women were randomized to either daily supplementation with 0.8 mg of folic acid or daily supplementation with a trace element for at least one month prior to conception and throughout the first two months of pregnancy. In the trace element group (n = 2391), 6.7 NTDs were expected; 6 NTDs were observed. In the folic acid group (n = 2471), 6.9 NTDs were expected; no participants in this group had an NTD-affected birth (Czeizel *et al*., 1994). These studies, along with several others, led the Centers for Disease Control and Prevention (CDC) to release the first guidelines regarding folic acid supplementation in pregnancy, recommending all women of childbearing age consume 0.4 mg of folic acid per day (Centers for Disease Control and Prevention, 1993).

2.2.5.2. Folate status and NTD risk

As folate deficiency became established as a risk factor for NTDs, and folic acid supplementation was realized to reduce this risk, Daly and colleagues aimed to evaluate the correlation between blood folate status and NTD risk (Daly *et al*., 1995). Between 1986 and 1990, blood samples were collected from 56,049 pregnant women in Ireland, and NTD cases among these pregnancies were ascertained following birth. Among the 81 pregnancies affected by NTDs, as well 247 healthy controls, both serum and RBC folate concentrations were analyzed and compared between groups to determine the incidence of NTDs at given

folate concentrations. Higher concentrations of both serum folate (Table 1) and RBC folate were found to correspond with significantly lower risks for NTD-affected pregnancies.

Table 1. Correlation of NTD-affected births and healthy controls with serum folate concentrations (Adapted from Daly *et al*.,1995)

Serum Folate (nmol/L)	No. of Cases (%)	No. of Controls (%)	NTD Risk per 1000 Births	95% CI
0 – 4.4	17 (21.0)	27 (10.9)	3.7	2.1-5.9
4.5 – 6.7	20 (24.7)	45 (18.2)	2.6	1.6-4.0
6.8 – 11.2	21 (25.9)	63 (25.5)	1.9	1.2-3.0
11.3 – 15.8	13 (16.0)	50 (20.3)	1.5	0.8-2.6
≥15.9	10 (12.4)	62 (25.1)	0.9	0.5-1.7
Total	**81 (100.0)**	**247 (100.0)**	**1.9**	**1.5-2.3**

Pregnant women achieving plasma folate concentrations greater or equal to 15.9 nmol/L were found to have a four-fold reduced NTD risk compared to those in the lowest plasma folate quantile, with a dose-response relationship observed along the range of folate status.

Using these data, along with 14 other studies investigating the effect of folic acid supplementation of serum folate status, Wald and colleagues estimated the effect of folic acid dose on increasing folate status and reducing the incidence of NTDs (Wald *et al*., 2001). Analysis of both placebo-controlled and uncontrolled studies found that a daily increase in folic acid intake of 0.1 mg was determined to increase steady-state serum folate concentrations by approximately 1.0 ng/mL (2.3 nmol/L). This assumption was used to extrapolate the expected reduction in NTD incidence based on an individual's baseline folate status and the respective increase in daily folic acid dose (Table 2).

Table 2. Expected reduction in NTD risk following various increased doses of folic acid supplementation, relative to baseline folate status (Adapted from Wald *et al*., 2001)

Increase in daily folic acid dose (mg)	NTD risk reduction expected per daily increase in folic acid supplementation			
	Baseline folate of 2.5 ng/mL	Baseline folate of 5.0 ng/mL	Baseline folate of 7.5 ng/mL	Baseline folate of 10.0 ng/mL
0.1	23%	13%	9%	7%
0.5	57%	41%	32%	27%
1.0	71%	57%	48%	41%
5.0	91%	85%	80%	75%

Ultimately, Wald and colleagues realized that the recommended 0.4 mg of folic acid per day only reduced the risk for NTDs by approximately 36%. As 5.0 mg would reduce the risk by 85% for the majority of individuals, they concluded that guidelines should be altered to reflect the potential for greater protection at a greater dose.

2.2.5.3. Folic acid fortification

Despite the realization that adequate folic acid supplementation during the periconceptional period could reduce the risk for NTDs, the incidence of these birth defects remained relatively high in the mid-1990s (Oakley *et al*., 1996). The majority of occurring NTD cases could be prevented by increasing folic acid intake, yet many women were not consuming sufficient amounts of the vitamin (Centers for Disease Control and Prevention, 1993). Accordingly, it was proposed that food products be fortified with folic acid, thus protecting women who fail to consume adequate amounts of folic acid prior to pregnancy,

as well as protect those who fail to supplement due to unplanned pregnancy (Wald and Bower, 1995).

As of January 1, 1998, fortification of cereal grain products became mandatory in the United States (Centers for Disease Control and Prevention, 2004). Canada followed suit soon after, fortifying flour with 150 µg of folic acid per 100 g, and pasta products with 240 µg of folic acid per 100 g (Turner and McCourt, 1998). These initiatives were expected to increase daily folic acid consumption by approximately 100 µg for women of childbearing age. This increased level of intake was considered optimal, as it was predicted to reduce the incidence of NTDs by over 20% without risking overexposure in certain groups (Daly *et al*., 1997). However, certain groups believed that further risk reduction with higher levels of fortification would be warranted (Wald *et al*., 1998).

Folic acid fortification of grain products quickly proved to be a successful public health initiative. In the United States, the prevalence of NTDs decreased by 19% in the year following mandatory fortification (Honein *et al*., 2001). By 2004, the CDC reported a drop in NTD rates by 26% compared to prior to fortification (Mills and Signore, 2004). In Canada, NTD rates decreased from 1.58 in 1000 births prior to fortification to 0.86 in 1000 births after fortification (De Wals *et al*., 2007). These decreases were apparent across all subtypes of NTDs (Table 3). Furthermore, the decreased prevalence was not attributed to increased compliance with supplementation recommendations, as these trends were apparent only in areas where fortification was implemented (Botto *et al*., 2006).

Table 3. Prevalence of NTDs (per 1000 births) before and after fortification, according to NTD subtype (Adapted from De Wals *et al*., 2007)

NTD Subtype	Pre-fortification	Post-fortification	Pre- to Post-Rate Ratio
Spina Bifida	0.86 (0.80-0.92)	0.40 (0.35-0.46)	0.47 (0.40-0.55)
Anencephaly	0.52 (0.45-0.58)	0.32 (0.24-0.38)	0.62 (0.52-0.74)
All Subtypes*	1.58 (1.48-1.64)	0.86 (0.80-0.92)	0.54 (0.49-0.60)

Results presented as rate (95% CI)

* Encephalocele, iniencephaly, and unspecified subtypes not shown

In concordance with these findings, indices of folic acid intake and blood folate status were shown to have increased during the post-fortification period. In the United States, folic acid consumption increased to approximately twice the predicted level, with daily intake ranging from 215 to 240 µg per day (Quinlivan and Gregory, 2003). Among Canadian women of childbearing age, RBC folate was found to have increased by an average of 214 nmol/L, from 527 nmol/L (95% CI 522-532) before fortification to 741 nmol/L (95% CI 737-744) afterwards (Ray *et al*., 2002).

Nonetheless, recent studies have found concerns regarding the current folate status of women of reproductive age. An analysis of blood samples among women aged 14-45 years old in Ontario identified 40% of non-pregnant women and 36% of pregnant women as having RBC folate concentrations below the 906 nmol/L (Bar-Oz *et al*., 2008). Similar concerns have been noted in surveys from the United States (Pfeiffer *et al*., 2007). Thus, even a decade after fortification, many women are still not achieving protective folate statuses either before or during pregnancy.

2.2.5.4. Current supplementation guidelines

Though much debate has surrounded the recommendations for sufficient folic acid supplementation prior to and after conception, official guidelines have not changed since the early 1990s. Both Health Canada and the CDC currently suggest that all women of childbearing age consume 0.4 mg of folic acid daily (Wilson *et al*., 2007, Centers for Disease Control and Prevention, 1993). More recent recommendations from other groups have expanded the guidelines, recommending up to 0.8 mg of folic acid, as well as a balanced diet containing natural folates (U.S. Preventative Services Task Force, 2009). Nonetheless, as most commonly used over-the-counter prenatal vitamins contain between 0.8-1.0 mg of folic acid, women who consume a prenatal multivitamin during the periconceptional period are often supplementing at or above the recommended amount.

For women with a previous history of NTDs, the American College of Obstetrics and Gynecology recommend a dose of 4.0 mg per day (U.S. Preventative Services Task Force, 2009), while the Society of Obstetricians and Gynaecologists of Canada (SOGC) recommend a dose of 5.0 mg per day (Wilson *et al*., 2007). Higher doses are additionally suggested to those taking anti-epileptic medications, patients with diabetes, certain ethnic groups, and obese individuals.

2.3. Obesity and Pregnancy

Maternal obesity during the periconceptional period is a growing public health issue, as the prevalence of obesity in the general population rises to unprecedented highs. Recent investigations into pregnancy-related complications associated with obese mothers have identified several concerns, each confounded by the multitude of comorbidities accompanying elevated weight.

2.3.1. Prevalence of obesity

Maternal obesity, as defined by a body mass index (BMI) greater than or equal to 30 kg/m^2, is a growing concern in Canada (Public Health Agency of Canada, 2009). Among non-pregnant women between the ages of 15-55 years old, 21.1% are defined as overweight (25.0-29.9 kg/m^2), with an additional 12.1% categorized as obese. As of 2005, the average pre-pregnancy BMI was 24.4 kg/m^2, near the upper end of the normal-weight range (18.5-24.9 kg/m^2). Approximately 13.6% of Canadian women were classified as obese during the pre-pregnancy period.

Statistics from the United States demonstrate an even higher prevalence of obesity among women of childbearing age. A comparison of data from the Pregnancy Risk Assessment Monitoring System between 1993 and 2003 found pre-pregnancy obesity rates increased from 13.0% to 22.0% (Kim *et al*., 2007). Data gathered from the 2003-2004 National Health and Nutrition Examination Survey (NHANES) indicated that 28.9% of women aged 20-39 years old were obese, with 8.0% approaching levels of morbid obesity (≥40 kg/m^2) (Ogden *et al*., 2006).

2.3.2. Obesity and neural tube defects

Maternal obesity was first realized as a risk factor for NTD-affected offspring nearly twenty years ago. In 1994, Waller and colleagues hypothesized that obesity's association with poor nutritional intake and metabolic disorders, themselves risk factors for NTDs, may underscore an elevated risk for these major malformations among obese pregnancies (Waller *et al*., 1994). To investigate this question, a case-control study of mothers with children affected by NTD and non-NTD malformations, as well those without any malformations, was conducted. Maternal weight was found to be significantly higher among the mothers with offspring affected by NTDs, as compared to mothers with healthy births (Table 4).

Table 4. Odds ratio of NTD-affected births according to maternal BMI (Adapted from Waller *et al*., 1994)

Body Mass Index (kg/m^2)	Neural Tube Defects (n)	Healthy Controls (n)	Odds Ratio	95% CI
< 16	3	4	0.8	0.2-3.8
16-17	24	23	1.2	0.7-2.1
18	36	44	0.9	0.6-1.5
19-27	360	403	Referent	-
28-30	28	34	0.9	0.5-1.6
31-37	32	20	1.8	1.0-3.2
≥ 38	16	6	3.0	1.2-7.7
Total	**499**	**534**	**-**	**-**

In the following two years, several more similar studies were conducted to corroborate these findings. Each of these examinations confirmed that pre-pregnancy obesity is associated with an elevated risk for NTD-affected births. Shaw and colleagues found an increased risk among mothers with a BMI greater than 29 kg/m^2, compared to those below that point (OR = 1.9, 95% CI 1.3-2.9) (Shaw *et al*., 1996). Watkins and colleagues arrived at nearly identical results, demonstrating a nearly 2-fold increased risk among obese mothers (OR = 1.92, 95% CI 1.08-3.40) (Watkins *et al*., 1996). Werler and colleagues identified the most substantially increased risk among mothers with a BMI greater than or equal to 32 kg/m^2 (RR = 2.8, 95% CI 1.1-6.7) (Werler *et al*., 1996). All studies found that the risk continued to rise as BMI approached levels of morbid obesity.

All three studies attempted to control for potential confounders, including maternal age, gravidity, education, use of alcohol or cigarettes, chronic illness, and folic acid supplementation. Interestingly, all studies found that even when controlling for folic acid intake among obese mothers, the risk for NTDs remained significantly higher. Additionally, none of the other variables were found to be responsible for this elevated risk among obese participants.

Since 1996, many other studies have substantiated these results and established maternal obesity as a risk factor for NTD-affected births (Kallen, 1998, Hendricks *et al*., 2001, Watkins *et al*., 2003, Anderson *et al*., 2005, Ray *et al*., 2005, Waller *et al*., 2007, Shaw and Carmichael, 2008, Oddy *et al*., 2009, Blomberg and Källén, 2010). Only three studies were located that did not find an association between maternal obesity and NTD risk. Feldman

and colleagues did not find an association between maternal weight and NTDs (Feldman *et al*., 1999). However, as weight, rather than BMI, was employed as the independent variable, it is possible that defining obesity according to BMI would elucidate an association. Moore and colleagues did not identify an elevated risk for NTDs among offspring born to obese mothers; they did, however, find an increased risk for various other defects, including orofacial clefts, hydrocephaly, and cardiac septal defects (Moore *et al*., 2000). Finally, Li and colleagues found a non-significant *decreased* risk for NTDs among obese individuals (Li *et al*., 2010). However, this study was conducted in the Shanxi Province of China, a region with a substantially higher prevalence of NTDs than is typically found in the Western world. Accordingly, it may be difficult for such a study to detect statistical differences according to maternal obesity.

Two recently published meta-analyses found similarly increased risks for NTD-affected births among obese mothers (Rasmussen *et al*., 2008, Stothard *et al*., 2009). Overall, maternal obesity was found to carry nearly a two-fold elevated risk, with a three-fold elevated risk found among those at the upper extremes of obesity. Both studies found commonly cited potential mechanisms for these findings, including metabolic disorders and nutritional deficiencies.

2.3.2.1. Obesity and folate status

As folate deficiency has been realized as one of the most established risk factors for NTDs, most studies investigating the association between obesity and these birth defects have noted this nutritional insufficiency as a potential linking mechanism. In fact, as many

of the malformations that arise from folate deficiency are also commonly associated with obesity (e.g. orofacial defects, cardiovascular anomalies), it is reasonably conceivable that a lower folate status may be implicated in their etiologies among obese individuals (Stothard *et al*., 2009). Indeed, poor nutritional intake is frequently found among obese populations. Consumption of fruit and vegetables has been found to be substantially lower among obese individuals (McNaughton *et al*., 2007). Elevated pre-pregnancy BMI negatively correlates with diet quality prior to pregnancy, and few positive alterations in diet are noted after beginning pregnancy (Laraia *et al*., 2007). De Jersey and colleagues found that among a sample of overweight and obese pregnant women, only 11% achieved folic acid supplementation meeting or exceeding an average of 400 µg per day (De Jersey *et al*., 2011). Other studies have additionally identified higher BMI as a predictor for failing to adequately supplement with folic acid during the periconceptional period (Goldberg *et al*., 2006). Accordingly, it would be unsurprising if reduced folate status played a role in the elevated NTD risk among obese mothers.

To account for this nutritional discrepancy, initial studies investigating the association between obesity and NTD risk controlled for folic acid intake. These studies found that, even after adjusting for folic acid consumption of at least 400 µg per day, the risk for NTDs remained significantly higher among obese mothers (Shaw *et al*., 1996, Watkins *et al*., 1996, Werler *et al*., 1996). More recent studies, conducted after fortification of grain products with folic acid, have arrived at similar findings (Ray *et al*., 2005, Waller *et al*., 2007, Oddy *et al*., 2009). However, while these studies controlled for intake of recommended amounts of folic acid, they did not examine whether participants reached

blood folate concentrations to reduce NTD risk. Watkins and colleagues acknowledged that obesity-associated metabolic alterations could have an impact on folate utilization or increase folate requirements (Watkins *et al*., 1996). Similarly, many pharmaceutical agents require weight-adjusted doses in overweight individuals, and thus, it is possible that the obese body necessitates an elevated folic acid intake to reach a protective blood folate status.

To evaluate if, even after controlling for folic acid intake, elevated BMI is associated with a reduced blood folate status, Mojtabai gathered data from two NHANES surveys; one prior to folic acid fortification in 1998 (NHANES III, n = 5018), and one several years after (NHANES 1999-2000, n = 1351) (Mojtabai, 2004). Only non-pregnant women of childbearing age (17-49 years old) were included, and both dietary and supplemented folate intake were taken into account. Results indicated that, both prior to and following fortification, elevations in BMI were associated with a decreased folate status (Table 5).

Table 5. Serum folate concentrations among participants in NHANES III and NHANES 1999-2000, categorized according to BMI (Adapted from Mojtabai, 2004)

	Serum Folate (ng/mL)				
Survey	**BMI <20.0**	**BMI 20.0-26.9**	**BMI 27.0-29.9**	**BMI ≥30.0**	***p*-value***
NHANES III	6.9 (6.1-7.7)	6.6 (6.1-7.0)	5.5 (4.9-6.1)	5.0 (4.6-5.3)	<0.001
NHANES 1999-2000	14.6 (11.9-17.3)	15.9 (14.7-17.2)	14.8 (12.8-16.8)	11.6 (11.0-12.3)	<0.001

BMI (kg/m^2), Results presented as mean (95% CI)

* Significance determined from statistical test for trend from regression coefficient associated with BMI variable

When adjusting for overall folate intake, multiple regression analysis found a significant decrease in serum folate concentrations for every 10 kg/m^2 increase in BMI (regression coefficient -0.153, SE 0.016, p <0.001). Ultimately, Mojtabai estimated that women in the highest BMI category (≥30.0 kg/m^2) would require an additional 350 μg of folic acid per day to reach the serum folate concentrations achieved by those in the lowest BMI category.

Other studies comparing obesity with serum folate status have largely found a negative correlation existing between the two variables. One study investigated serum vitamin levels in a sample of overweight and obese Thai participants, and found significantly lower serum folate concentrations in both groups compared to normal-weight controls (Tungtrongchitr *et al*., 2003). Another study obtained baseline serum folate concentrations prior to randomizing participants to one of two weight-loss diets, and found obese individuals to be approximately 6-fold more likely to have folate levels under 14.9 nmol/L (Ortega *et al*., 2009). Most notably, this group found that serum folate concentrations increased significantly among those who lost more weight, despite overall intake remaining unchanged, indicating that body size may have an impact on circulating vitamin levels.

2.3.2.2. Obesity and other confounders

Aside from folate deficiency, other obesity-associated factors have been proposed to potentially mediate the increased NTD risk in this population. Diabetes mellitus and gestational diabetes have both been well-established as risk factors for NTD-affected births (Becerra *et al*., 1990, Anderson *et al*., 2005). However, two of the initial studies investigating obesity and the risk for NTDs adjusted for participants with diabetes, and the

risk remained statistically significant (Shaw *et al*., 1996, Werler *et al*., 1996). Additionally, Watkins and colleagues excluded participants with metabolic disorders from their study (Watkins *et al*., 1996).

Detection issues in obese mothers have also been suggested as an impediment to recognizing NTD-affected fetuses, thus reducing terminations of pregnancies with infants affected by these malformations. Ultrasound visualization of the fetus is significantly impaired for individuals in the upper BMI percentiles (Wolfe *et al*., 1990), especially during the early weeks of pregnancy (Hendler *et al*., 2004). Nearly half of fetal anatomy ultrasounds conducted at 18 weeks in obese mothers result in suboptimal visualization (Dashe *et al*., 2009). However, a meta-analysis conducted by Stothard and colleagues noted no change in the elevated risk for NTDs among obese individuals when only studies that included pregnancy terminations were considered (Stothard *et al*., 2009). Additionally, such a bias would skew NTD detection in obese mothers to post-birth, though recent analyses have found a similar pre- to post-natal detection ratio among NTD cases for both obese and non-obese mothers (Waller *et al*., 2007).

Genetic differences associated with obesity have additionally been presented as a theory for the elevated risk for NTDs. The 677C→T polymorphism of the MTHFR is commonly found in the general population, resulting in reduced enzymatic efficiency at regular homeostatic temperatures (Zhu *et al*., 2009). Accordingly, individuals with the homozygous variant of this polymorphism are at risk for reduced folate status (Bailey and Gregory, 1999), and, according to some studies, an increased risk for NTD-affected births

(van der Put *et al*., 1995). Indeed, a small meta-analysis confirmed a nearly two-fold elevated risk among either mothers or NTD-patients with this MTHFR polymorphism (van der Put *et al*., 1997). These findings have led others to investigate whether this genetic variant may be associated with the reduced folate status among obese individuals, thus accounting for the elevated risk for NTDs. However, a combination of three large population studies did not confirm an association between BMI and the 677C→T MTHFR polymorphism (Lewis *et al*., 2008).

2.3.3. Obesity and folate status: mutually exclusive risk factors?

As previously mentioned, obesity has been linked to folate deficiency, even after adjusting for overall folate intake. These assessments have led to certain recommendations regarding elevated folic acid doses for obese women of childbearing age (Mojtabai, 2004). In 2007, the SOGC, in association with the Motherisk program, released multivitamin supplementation guidelines for pre-conception care (Wilson *et al*., 2007). These guidelines suggested that individuals with a BMI greater than 35 kg/m^2 supplement with 5.0 mg of folic acid for three months prior to conception, and continuing throughout the first trimester of pregnancy. While these recommendations were based on the knowledge that folate deficiency associated with this at-risk population can increase the risk for NTDs, no studies have yet confirmed that an elevated folic acid dose can effectively reduce the risk for major malformations among infants born to these individuals. Furthermore, no investigations have yet been undertaken to ensure that an elevated dose will lead to protective serum folate concentrations. Accordingly, it remains unknown if obesity and folate deficiency are mutually exclusive risk factors for NTDs.

CHAPTER 3. MATERIALS & METHODS

3.1. Comparison of folic acid pharmacokinetics in obese and non-obese women of childbearing age

3.1.1. Use of previously published data

The current study incorporated data previously collected by our group from two published studies investigating folic acid pharmacokinetics following oral administration of either a 1.1 mg (Ahn *et al*., 2005) or 5.0 mg (Nguyen *et al*., 2008) dose to healthy women of childbearing age. In each of these studies, six participants received a folic acid dose (as part of a prenatal multivitamin formulation) following a baseline blood sample, and eight subsequent blood samples were obtained over the course of a 10-hour period. Serum folate concentrations were analyzed, and area under the concentration-time curve (AUC), as well as additional pharmacokinetic variables, was calculated from the concentration-time curve.

For the current study, we aimed to compare the single-dose folic acid pharmacokinetics of obese (n = 12) and non-obese (n = 12) non-pregnant women of childbearing age. Of the twelve participants from the previous studies, information regarding participant height (in the calculation of BMI) was unavailable for three women, and one woman was classified as 'overweight' (BMI 25.0-29.9 kg/m^2). Accordingly, data was available for three obese and five non-obese participants for inclusion in the current study. An additional nine obese and seven non-obese participants were required to fulfill the sample size requirement.

3.1.2. Study recruitment

Participants were recruited from October 2009 to December 2009 using posters within The Hospital for Sick Children (Toronto, Canada) and online postings within the University of Toronto's intranet. Upon meeting study eligibility, participants were provided with the study protocol and signed a consent form (Appendix A), in accordance with and approved by the Ethics Research Board of the hospital.

3.1.3. Inclusion and exclusion criteria

Current study eligibility criteria were devised in concordance with those used in the previous studies. Participants were eligible for study inclusion if they met the criteria for healthy women of childbearing age. Childbearing age was defined as 18-45 years old. Obesity and non-obesity were defined according to the World Health Organization's International Classification of BMI (obese, BMI ≥30 kg/m^2; non-obese, BMI 18.50-24.99 kg/m^2).

Recruited women were ineligible for participation if they had used a folic acid-containing multivitamin in the previous 6 months, or if they were using any medication that could interfere with folic acid pharmacokinetics (e.g. valproic acid or methotrexate). Additional exclusion criteria included any chronic medical conditions.

3.1.4. Participant pairing

To account for weight differences among the obese and non-obese participants, participants were administered weight-adjusted folic acid doses. Each newly recruited

participant (C) was matched to a participant from the previous studies (P), so as to create twelve obese/non-obese pairs (Figure 4). Folic acid doses for each current participant were calculated from previous participants by dividing the administered folic acid dose by the previous participant's total body weight, and then multiplying by the current participant's total body weight:

$$Dose_C = {}^{Dose_P}/_{Weight_P} \times Weight_c$$

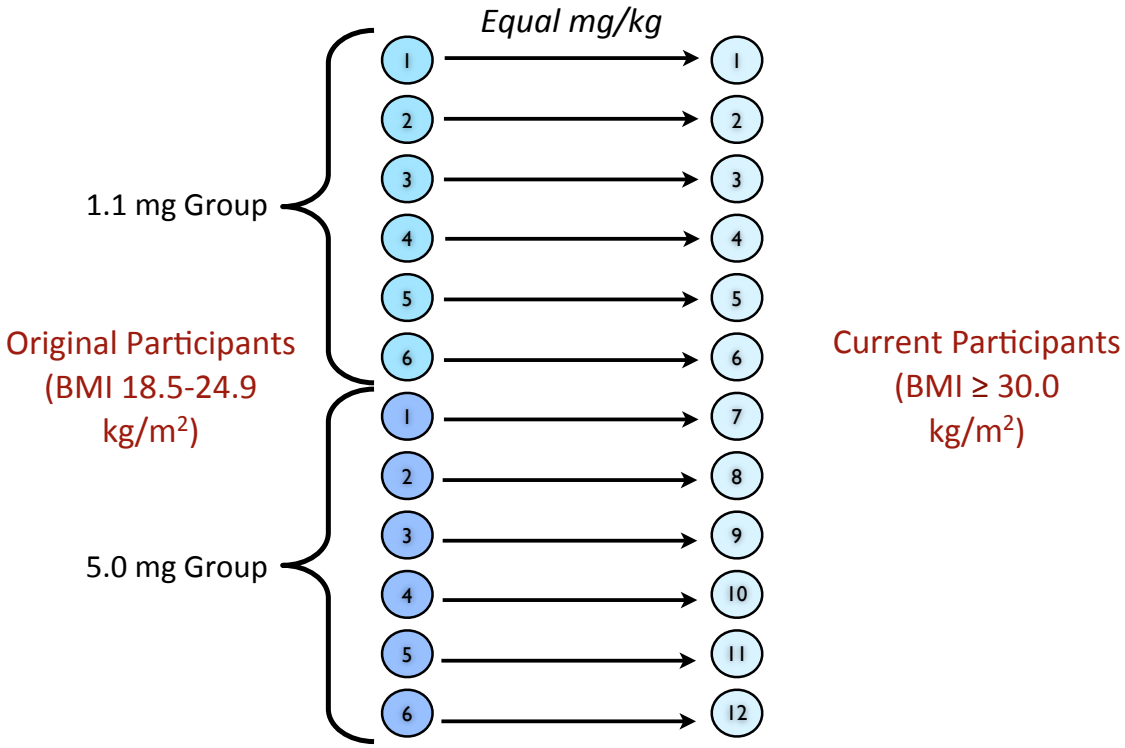

Figure 4. Simplified schematic representation of participant pairing between studies

Newly recruited obese participants were each paired to an initial participant from either the 1.1 mg or 5.0 mg group. Obese participants were administered folic acid doses equivalent to the mg/kg dose received by their respectively paired non-obese participant.

Calculated doses were created by scoring PregVit® (1.1 mg) and PregVit-Folic 5® (5.0 mg) prenatal vitamins (Duchesnay Inc., Blainville, Quebec).

3.1.5. Study protocol

After a minimum 6-hour fast, participants arrived in the morning for a baseline blood sample. An intravenous line was inserted to avoid repeated venipunctures. Immediately after baseline blood sampling, participants were orally administered the respective folic acid dose. Subsequent blood samples were taken at 0.5, 1, 2, 3, 4, 6, 8, and 10 hours post-administration. Blood was collected in BD Vacutainer® tubes (Becton, Dickinson and Co., Franklin Lakes, New Jersey) with a gel for serum separation. Each participant was given a standardized meal throughout the study day containing a known amount of dietary folate (0.06 mg).

Blood samples were left to clot at room temperature for 30 minutes after collection. Samples were then centrifuged at 4°C for 15 minutes at 2500 rpm. Serum was collected and frozen at -20°C until analysis. All samples were analyzed as a batch using the Access Folate assay system (Beckman Coulter Inc., Fullerton, CA). This technique utilizes a competitive binding receptor assay to quantify serum folate, including that which is bound to endogenous binding proteins. The method has a lower limit of detection at 0.5 ng/mL. Samples above the linear range were diluted to bring them within the linear range of the assay.

Concentration-time curves were generated for each participant using the serum folate concentrations plotted against the time of blood draw. AUC (nmol·h/L) was calculated using the trapezoidal rule and normalized to baseline folate concentrations. C_{max} (nmol/L) and t_{max} (h) were derived directly from the concentration-time curves. k_{el} (h^{-1}) was

derived from the logarithmic linear segment of the curve after C_{max} had been reached. $t_{1/2}$ (h) was subsequently calculated as $^{\ln 2}/_{k_{el}}$, and V_d (L) was calculated as $^{Dose}/_{(AUC \times k_{\mathrm{el}})}$. CL (mL/min) was calculated by dividing the dose by the AUC. When necessary, measurements calculated as ng/mL were converted to nmol/L by multiplying by a factor of 2.27. Milligrams were converted to nanomoles by dividing by a factor of 0.000441.

LBW was used to explain AUC differences between obese and non-obese participants. Due to limitations of the initial lean body weight (LBW) equation for individuals in the upper categories of obesity (Han *et al*., 2007), the 2005 revised LBW equation was used (Janmahasatian *et al*., 2005),

$$LBW\ (kg) = {}^{(9270 \times kg_{TBW})}\Big/_{[8780 + (244 \times BMI)]}$$

where total body weight (TBW) is given in kilograms.

3.1.6. Statistical analysis

Statistical analyses were performed using the SPSS package (SPSS, Version 17, Chicago, Illinois). Descriptive statistics were used to describe patient characteristics and pharmacokinetic parameters. The obese group was compared to the non-obese group by means of chi-squared test or Fisher's exact test for differences in categorical variables. Differences between obese and non-obese pairs were analyzed between normal-weight and obese participants using the Wilcoxon signed-rank test. Simple linear regression was employed to correlate dose standardized to both TBW and LBW with AUC.

3.2. Guidelines for folic acid supplementation – accounting for lean body weight in women of childbearing age

As LBW was found to be the strongest predictor of systemic exposure to folic acid, we intended to estimate the daily dose required to achieve folate concentrations ≥15.9 nmol/L in women across a wide range of BMIs, tailored to their height and weight. This would further elucidate whether obese women of childbearing age necessitate a higher dose than is currently recommended to achieve and maintain steady-state serum folate levels equal to or greater than 15.9 nmol/L.

3.2.1. Calculation of folic acid dose per lean body weight

To assess folic acid dosing requirements according to LBW, pharmacokinetic data from the completed study comparing obese and non-obese women of childbearing age were used. Steady-state serum folate concentrations were estimated using the equation, $(F \times Dose) \Big/ (CL \times \tau)$, where τ represents dosing intervals. Bioavailability (F) was assumed to be 100%. Steady-state levels were plotted against the dose received per kilogram LBW. Linear regression analysis was used to identify the dose per LBW that would result in reaching steady-state serum folate levels of ≥15.9 nmol/L.

3.2.2. Estimation of daily folic acid dose in achieving serum folate concentrations to reduce NTD risk

LBW was calculated for women across a wide range of BMI values. The dose per LBW expected to lead to protective steady-state levels was multiplied by the calculated LBWs.

CHAPTER 4. RESULTS

4.1. Comparison of folic acid pharmacokinetics between obese and non-obese women of childbearing age

A total of twenty-four participants were included in the current study, incorporating data from both newly recruited participants (n = 16) and from those who had completed the previous studies (n = 8). Participant characteristics by group are presented in Table 6.

Table 6. Participant characteristics according to obese and non-obese study groups

	Non-Obese Group (n=12)	Obese Group (n=12)	*p*-value
Age (y)	25.6 ± 4.0	37.2 ± 5.4	<0.001
Height (m)	1.62 ± 0.07	1.68 ± 0.06	0.05
BMI (kg/m^2)	22.7 ± 1.4	34.6 ± 5.5	0.002
TBW (kg)	59.8 ± 5.9	99.4 ± 17.5	0.002
Gravidity			
0	12	2	<0.001
1	0	1	
2	0	8	
3	0	1	
Race			
White	7	9	0.67
Black	0	2	
Asian	5	1	
Employment			
Full-time	3	11	0.004
Student	8	1	
Unemployed	1	0	
Reported smoking	1	4	0.64
Reported alcohol	4	2	0.32

Data are presented as Mean ± Standard Deviation
BMI. body mass index; TBW, total body weight

As expected, TBW and BMI were found to be significantly higher in the obese group (99.4 ± 17.5 kg and 34.6 ± 5.5 kg/m^2, respectively) than the non-obese group (59.8 ± 5.9 kg and 22.7 ± 1.4 kg/m^2, respectively), though height was similar between the groups. The mean age of the obese group was found to be significantly higher than that of the non-obese group (37.2 ± 5.4 years vs. 25.6 ± 4.0 years, p <0.001, respectively). Additionally, significant differences between groups were detected for both gravidity and employment status, though neither of these factors was expected to have an impact on study outcomes. Race, reported smoking, and reported alcohol use were found to be similar between the two groups.

To assess differences in folic acid pharmacokinetics between the groups, a concentration-time curve was generated for each participant, and pharmacokinetic variables were derived from each curve. Concentration-time curves for both non-obese and obese groups are shown in Figure 5 and Figure 6, respectively. Group differences for these variables are presented in Table 7.

No differences in baseline serum folate were found between the obese and non-obese groups. As the obese participants received an equivalent dose of folic acid per kilogram body weight as their respective non-obese pairs, the mean absolute dose administered was higher in the obese group [3.6 mg (range 1.5-12.5 mg) vs. 2.3 mg (range 1.1-5.0 mg), p = 0.002)]. The administered doses per kilogram TBW ranged from 0.0153 to 0.0917 mg/kg.

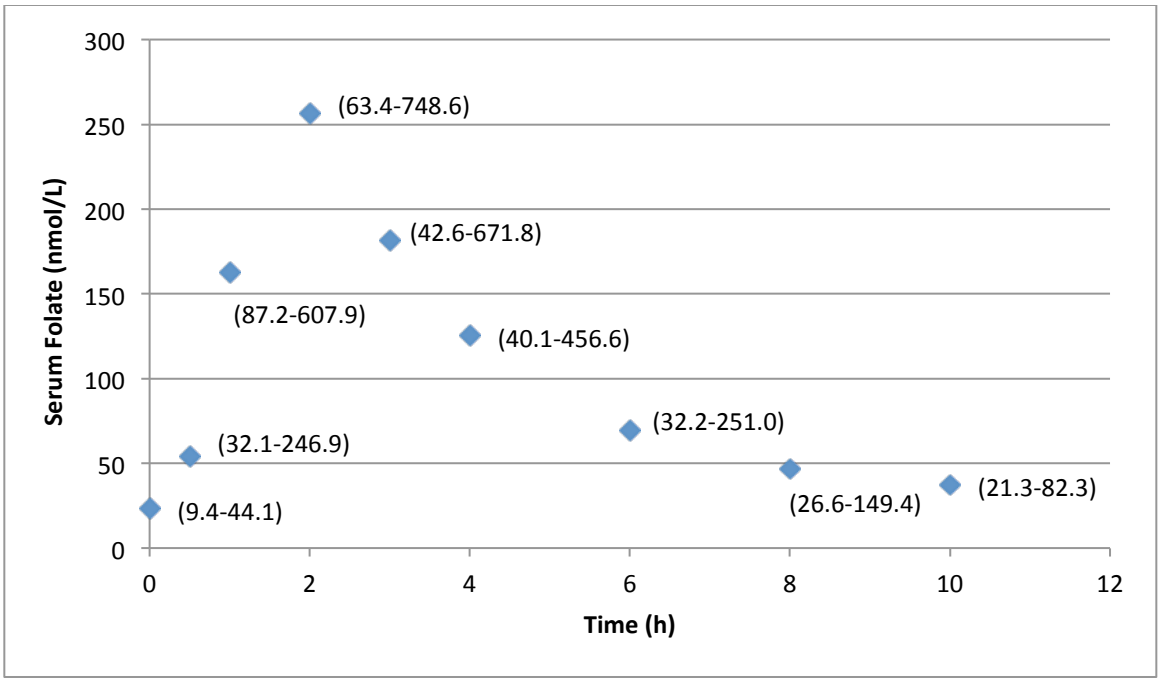

Figure 5. Concentration-time curve for non-obese group

Data are presented as Median (Range)

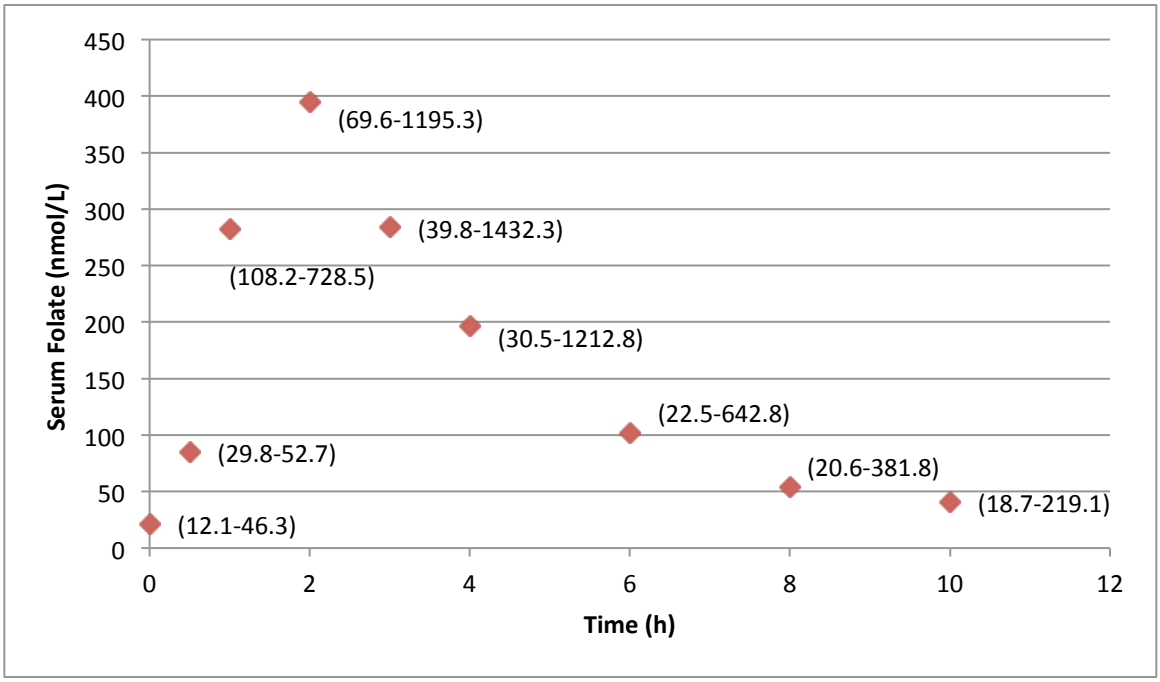

Figure 6. Concentration-time curve for obese group

Data are presented as Median (Range)

Table 7. Comparison of single-dose folic acid pharmacokinetics between obese and non-obese study groups

	Non-Obese Group (n=12)	Obese Group (n=12)	*p*-value
Baseline folate (nmol/L)	23.1 (9.4-44.1)	21.6 (12.1-46.3)	0.94
Dose (mg)	2.3 (1.1-5.0)	3.6 (1.5-12.5)	0.002
AUC (nmol·h/L)	981.2 (156.5-2963.5)	1482.3 (228.4-6724.1)	0.008
$t_{1/2}$ (h)	2.28 (1.83-3.02)	2.42 (1.94-2.91)	0.75
t_{max} (h)	1.0 (1.0-2.0)	1.0 (1.0-3.0)	0.56
C_{max} (nmol/L)	301.3 (87.2-748.6)	447.4 (108.2-1432.3)	0.01
V_d (L)	17.4 (12.1-56.5)	18.9 (13.9-63.8)	0.30
CL (mL/min)	87.9 (59.6-265.3)	90.7 (69.9-252.9)	0.31

Data are presented as Median (Range)
AUC, area under the curve; $t_{1/2}$, half-life; t_{max}, time to maximum concentration; C_{max}, maximum concentration reached; V_d, volume of distribution; CL, total apparent clearance

Overall systemic folic acid exposure, as assessed by AUC, was analyzed as the primary outcome. AUC was found to be significantly larger among the obese participants [1482.3 nmol·h/L (range 228.4-6724.1 nmol·h/L) vs. 981.2 nmol·h/L (range 156.5-2963.5 nmol·h/L), p = 0.008], with a corresponding elevation in C_{max} (p = 0.012). No differences were detected for $t_{1/2}$, t_{max}, V_d, or CL between the groups.

4.2. Standardization of results according to lean body weight

To explain larger AUC values in the obese group as compared to the non-obese group, LBW was investigated as a potential determinant of folic acid exposure. Administered folic acid doses were standardized according to both TBW and LBW. Results from this subsequent analysis are presented in Table 8.

Table 8. Normalization of folic acid dose according to body weight measurement

	Non-Obese Group (n=12)	Obese Group (n=12)	*p*-value
LBW (kg)	38.6 (34.9-44.9)	55.0 (42.7-61.9)	0.002
LBW/TBW (%)	64.9 (62.4-67.6)	55.7 (45.4-57.6)	0.002
Dose (mg)	2.3 (1.1-5.0)	3.6 (1.5-12.5)	0.002
Dose/TBW (mg/kg_{TBW})	0.035 (0.015-0.092)	0.035 (0.015-0.092)	-
Dose/LBW (mg/kg_{LBW})	0.054 (0.025-0.143)	0.065 (0.027-0.202)	0.002

Data are presented as Median (Range)
LBW, lean body weight; TBW, total body weight

Despite its use as a weight measurement for non-adipose tissues, LBW was found to be significantly higher in the obese group [55.0 kg (range 42.7-61.9 kg) vs. 38.6 kg (range 34.9-44.9 kg), p = 0.002], albeit the differences between groups were less prominent than those of TBW. LBW as a percentage of TBW was found to decrease in the obese group (p = 0.002). Accordingly, though the folic acid dose administered per kilogram TBW was identical between the groups, relative doses were found to be higher in the obese group after normalizing to milligram per kilogram LBW (p = 0.002).

Consequently, AUC was compared as a function of either dose per TBW (Figure 7) or dose per LBW (Figure 8) to identify the dosing measurement that more accurately predicts overall systemic exposure to folic acid. Defining AUC as a function of dose per LBW was found to have a significantly stronger association than as a function of dose per TBW (R^2 = 0.90 vs. R^2 = 0.76, respectively, p <0.001).

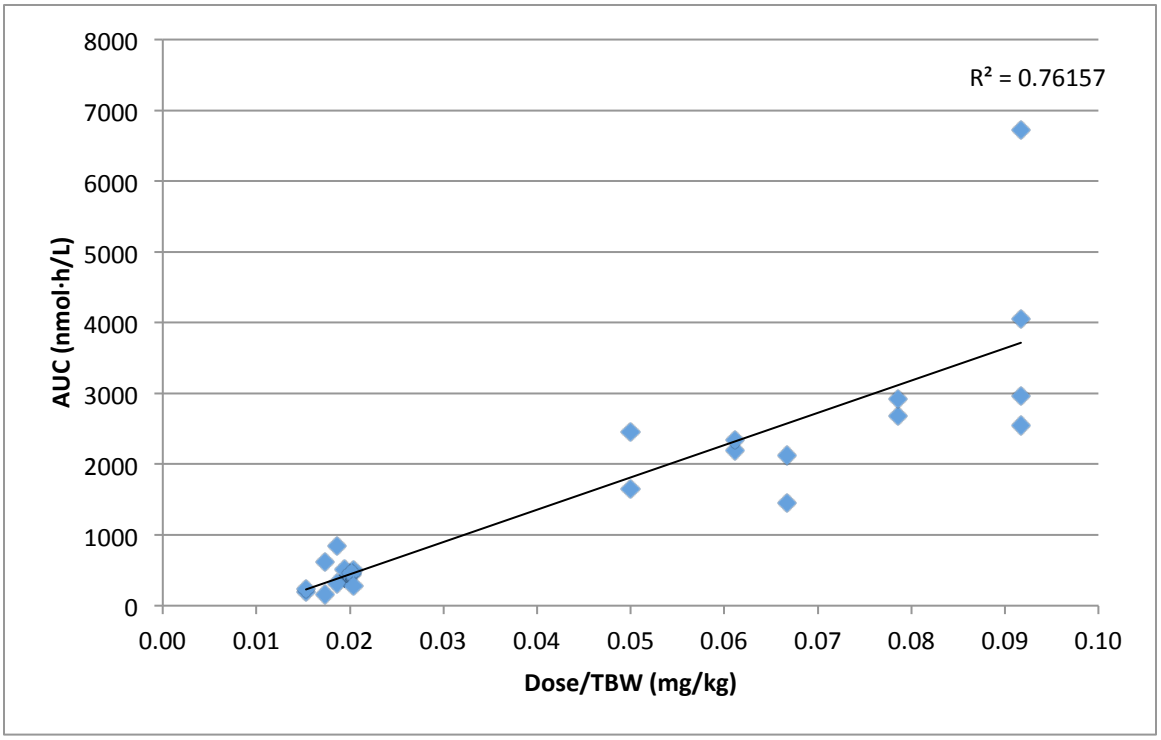

Figure 7. Correlation of folic acid dose per kilogram TBW with AUC

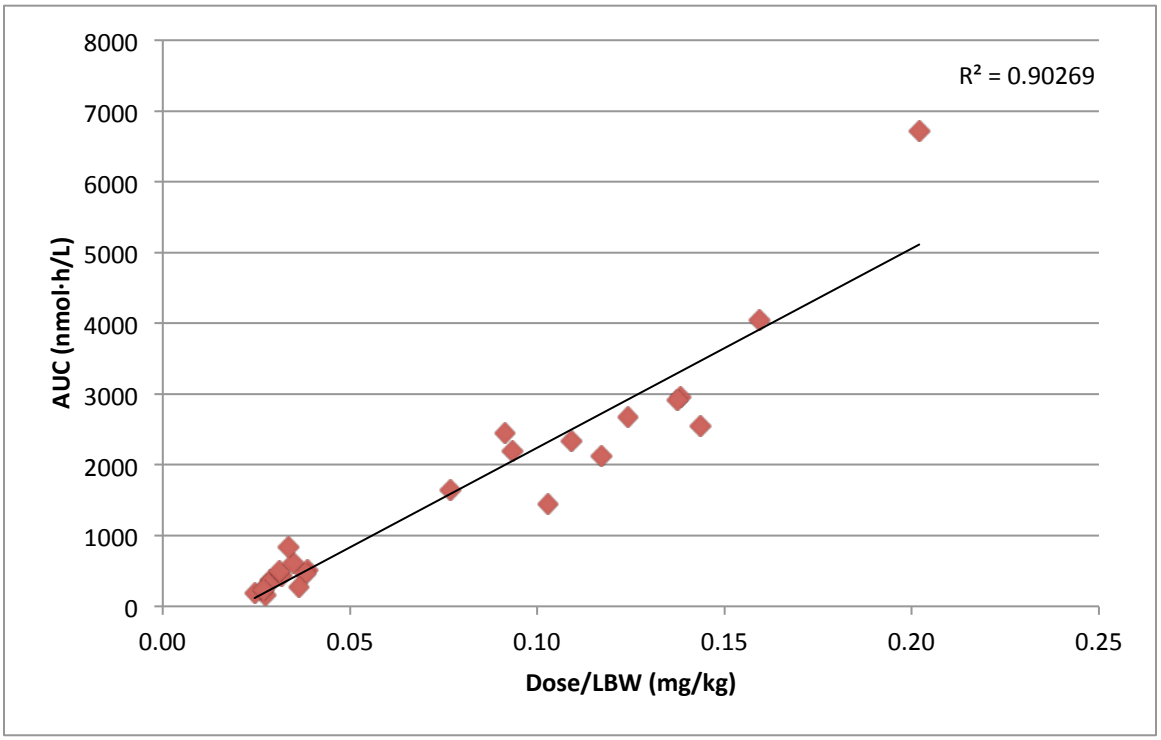

Figure 8. Correlation of folic acid dose per kilogram LBW with AUC

4.3. Estimation of daily folic acid dose in achieving serum folate concentrations to reduce NTD risk

As the folic acid dose per kilogram LBW was found to be a stronger predictor of overall systemic exposure than dose per kilogram TBW, we intended to estimate the daily dose per LBW that would be expected to achieve steady-state serum folate levels of ≥15.9 nmol/L. Steady-state serum folate levels were calculated for each participant at their respective doses, and plotted against the dose per kilogram LBW that they received (Figure 9).

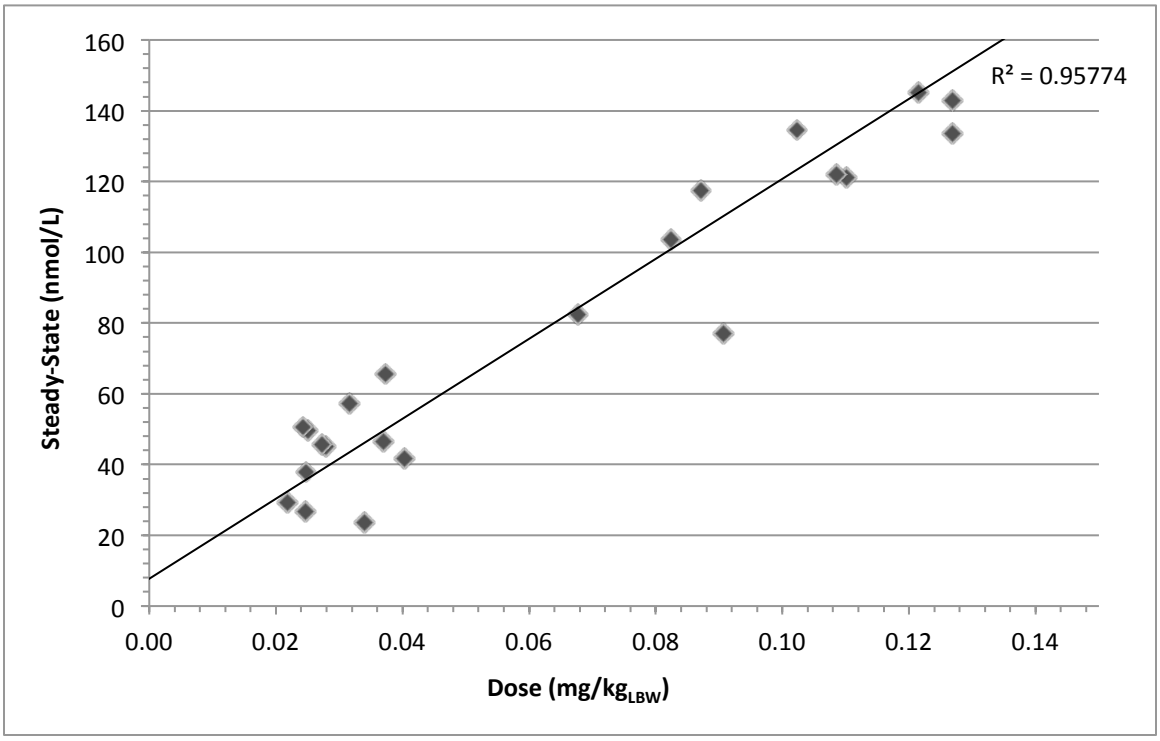

Figure 9. Correlation of dose per kilogram LBW with estimated steady-state serum folate concentrations

The dose per kilogram LBW was found to strongly correlate with estimated serum folate levels (R^2 = 0.96). Using this regression line, the average dose per kilogram LBW that

would be expected to result in steady-state serum folate levels of ≥15.9 nmol/L was identified as 0.0073 mg/kg_{LBW}.

To understand how the estimated daily dose would differ for women across a wide span of BMI values, a chart was created for individuals at various weights and heights within this BMI range. After calculating the LBW for each individual at a given weight and height, the LBW was multiplied by 0.0073 mg/kg_{LBW} to estimate the required folic acid dose (Table 9).

Table 9. Daily doses of folic acid (mg) estimated to maintain serum folate concentrations at ≥15.9 nmol/L

Height (m) \ Weight (kg)	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	135	140	145	150
1.44	0.21	0.23	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.32	0.33	0.34	0.34	0.35	0.36	0.36	0.37	0.37	0.37	0.38
1.46	0.22	0.23	0.24	0.26	0.27	0.28	0.29	0.30	0.31	0.31	0.32	0.33	0.34	0.34	0.35	0.36	0.36	0.37	0.37	0.38	0.38	0.39
1.48	0.22	0.23	0.25	0.26	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.34	0.35	0.36	0.36	0.37	0.37	0.38	0.38	0.39	0.39
1.50	0.22	0.23	0.25	0.26	0.27	0.29	0.30	0.31	0.32	0.32	0.33	0.34	0.35	0.35	0.36	0.37	0.37	0.38	0.38	0.39	0.39	0.40
1.52	0.22	0.24	0.25	0.26	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.35	0.36	0.37	0.37	0.38	0.39	0.39	0.40	0.40	0.41
1.54	0.22	0.24	0.25	0.27	0.28	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.37	0.38	0.39	0.39	0.40	0.40	0.41	0.41
1.56	0.23	0.24	0.26	0.27	0.28	0.30	0.31	0.32	0.33	0.34	0.35	0.35	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.41	0.42	0.42
1.58	0.23	0.24	0.26	0.27	0.29	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.38	0.39	0.40	0.40	0.41	0.42	0.42	0.43
1.60	0.23	0.25	0.26	0.28	0.29	0.30	0.31	0.33	0.34	0.35	0.36	0.36	0.37	0.38	0.39	0.40	0.40	0.41	0.42	0.42	0.43	0.43
1.62	0.23	0.25	0.26	0.28	0.29	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.39	0.40	0.41	0.42	0.42	0.43	0.43	0.44
1.64	0.23	0.25	0.27	0.28	0.30	0.31	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.41	0.42	0.43	0.44	0.44	0.45
1.66	0.24	0.25	0.27	0.28	0.30	0.31	0.32	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.40	0.41	0.42	0.43	0.43	0.44	0.45	0.45
1.68	0.24	0.25	0.27	0.29	0.30	0.32	0.33	0.34	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.43	0.44	0.45	0.45	0.46
1.70	0.24	0.26	0.27	0.29	0.30	0.32	0.33	0.34	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.42	0.43	0.44	0.45	0.45	0.46	0.47
1.72	0.24	0.26	0.28	0.29	0.31	0.32	0.33	0.35	0.36	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.44	0.45	0.46	0.47	0.47
1.74	0.24	0.26	0.28	0.29	0.31	0.32	0.34	0.35	0.36	0.37	0.39	0.40	0.41	0.42	0.43	0.43	0.44	0.45	0.46	0.47	0.47	0.48
1.76	0.24	0.26	0.28	0.30	0.31	0.33	0.34	0.35	0.37	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.46	0.47	0.48	0.49
1.78	0.25	0.26	0.28	0.30	0.31	0.33	0.34	0.36	0.37	0.38	0.39	0.40	0.42	0.43	0.44	0.44	0.45	0.46	0.47	0.48	0.49	0.49
1.80	0.25	0.27	0.28	0.30	0.32	0.33	0.35	0.36	0.37	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.48	0.49	0.50
1.82	0.25	0.27	0.29	0.30	0.32	0.34	0.35	0.36	0.38	0.39	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.50
1.84	0.25	0.27	0.29	0.31	0.32	0.34	0.35	0.37	0.38	0.39	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.50	0.51
1.86	0.25	0.27	0.29	0.31	0.32	0.34	0.36	0.37	0.38	0.40	0.41	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52
1.88	0.25	0.27	0.29	0.31	0.33	0.34	0.36	0.37	0.39	0.40	0.41	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.52
1.90	0.25	0.27	0.29	0.31	0.33	0.35	0.36	0.38	0.39	0.40	0.42	0.43	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53
1.92	0.26	0.28	0.30	0.31	0.33	0.35	0.36	0.38	0.39	0.41	0.42	0.43	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54
1.94	0.26	0.28	0.30	0.32	0.33	0.35	0.37	0.38	0.40	0.41	0.42	0.44	0.45	0.46	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54
1.96	0.26	0.28	0.30	0.32	0.34	0.35	0.37	0.39	0.40	0.41	0.43	0.44	0.45	0.47	0.48	0.49	0.50	0.51	0.52	0.53	0.54	0.55
	Underweight						**Normal Weight**					**Overweight**				**Obese**						

Underweight, BMI <18.5 kg/m^2; Normal-Weight, BMI 18.5-24.9 kg/m^2; Overweight, BMI 25.0-29.9 kg/m^2; Obese, BMI ≥30 kg/m^2

CHAPTER 5. DISCUSSION

5.1. Characteristics of the Study Population

The participants recruited for this study were identified through posters with The Hospital for Sick Children and online postings within the University of Toronto's intranet. As a consequence of these recruitment methods, the non-obese participant group was markedly younger and exclusively nulligravida. Differences in age were not expected to have any effect on results, as previous work conducted by our group did not identify any differences in any pharmacokinetic parameters for folic acid across an age range from 24 to 44 years old (Nguyen *et al*., 2008). Similarly, differences in gravidity were not expected to affect pharmacokinetic variables. While, in theory, fluid expansion associated with pregnancy could potentially dilute serum concentrations following folic acid administration, the fact that plasma volumes return to normal following birth (Davison and Lindheimer, 1989) would preclude this as a confounder.

The baseline folate status of the recruited participants was found to be similar to those identified in other post-fortification population-based surveys (Mojtabai, 2004, Ray *et al*., 2007). Of the twenty-four total participants, only three non-obese and two obese participants had baseline serum folate status below the concentration of 15.9 nmol/L. However, unlike studies that have identified a moderately lower serum folate status in obese individuals, no significant differences were found between our study groups. This may be attributed to the limited sample size in our study.

5.2. Folic acid dose per lean body weight as strongest predictor of folic acid exposure

Our current study sheds light on folic acid handling in obese women of childbearing age, when compared to those of normal-weight. Despite equivalent dosing per kilogram TBW, AUC levels were found to be higher in the obese group. This weight-adjusted method of dose administration was chosen due to the fact that previous studies had already identified that, when controlling for absolute folate intake, obese women exhibited lower serum folate status. Additionally, many pharmacological agents are prescribed on a per kilogram dosing regimen, as the size of the body must be considered in order to ensure that sufficient systemic concentrations are maintained.

Theoretically, using this weight-adjusted dosing between pairs should lead to similar systemic folic acid exposure. However, the elevated AUC in obese individuals, with no differences in total apparent clearance, would indicate a likelihood that folic acid does not freely distribute into adipose tissue. Consequently, it would appears that AUC differences could be explained by the administered dose of folic acid remaining within a smaller area than the TBW.

When initially investigating body measurement-related determinants of AUC, LBW was considered due to its relationship with water-soluble drugs (Green and Duffull, 2004). Defined as any body weight consisting of extracellular fluid, skeletal muscle, bone, and vital organs (Janmahasatian *et al*., 2005), LBW is notable in that 99% of metabolic processes occur within these tissues (Roubenoff and Kehayias, 1991). Folic acid plays an integral role in several intracellular anabolic processes, by means of one-carbon transfer, including: a)

conversion of deoxyuridine to deoxythymidine, b) synthesis of purine bases, and c) remethylating homocysteine to methionine. As these activities are vital for the formation and methylation of DNA, activities frequently occurring in lean tissues, it was hypothesized that systemic exposure to administered folic acid may be more strongly tied to the participants' LBW than TBW.

When comparing an obese individual to a non-obese individual in terms of body composition, it is often assumed that the sole difference between the two is the extent of adipose tissue. However, studies have identified alterations in body composition that occur secondary to a rise in levels of fat mass. As early as 1859, researchers have found an increase in LBW that occurs following a rise in adipose tissue. One-third of the body weight put on by overfed animals prior to being sold was reported to be LBW (Lawes and Gilbert, 1859). Subsequent investigations identified several facets of obesity that would be indicative of a rise in LBW in addition to adipose tissue. Creatinine output, which is quantitatively tied to the extent of LBW, was found to increase with TBW in obese children (Kahn and Smith, 1936). The authors further noted that in every case of obesity, the increase in weight is "due as much to increase of muscle as to increase of fat". An ensuing study identified an increase in basal metabolic rate in obesity correlated with the percentage of excess body weight (Bruch, 1939). Similarly, while glomerular filtration rates are typically higher in obese individuals, normalizing these values to LBW removes such differences (Janmahasatian *et al*., 2008).

More recent work by Forbes confirmed the association between LBW and obesity. Longitudinal follow-up of children who gained weight during childhood found a moderate increase in height (due to LBW) exclusively following an increase in adipose tissue (Forbes, 1977). Studies of children and adults, with both established obesity and that due to overfeeding, found an elevated LBW in obese individuals compared to non-obese when matching for age, sex, and height (Forbes and Welle, 1983). LBW was found to account for up to 40% of the excess body weight in these individuals.

Initially, the original LBW equation for females, as derived in 1994, was used in the calculation for our study participants (Morgan and Bray, 1994):

$$LBW\ (kg) = (1.07 \times TBW) - (0.0148 \times BMI \times TBW)$$

However, use of this equation resulted in a lower LBW for the most obese individuals, compared to those in the mid-range of obesity. It was then realized that, due to the limited range of BMI values for individuals used in developing this initial LBW equation, LBW values begin to decline for individuals with a BMI above approximately 34 kg/m^2 (Green and Duffull, 2002). In 2005, Janmahasation and colleagues established a new equation, standardized to a sample of individuals with a broader BMI range (17.1-69.9 kg/m^2) (Janmahasation *et al*., 2005). The revised 2005 equation was subsequently used for calculation of LBW. Accordingly, applying the LBW equation to obese and non-obese participants identified a significantly higher LBW in the obese participants, albeit the gap between the groups was much less pronounced than that of TBW.

As such, the administered dose of folic acid, while matched per kilogram TBW between pairs, was in fact higher per kilogram LBW among obese participants. Upon correlating both the folic acid dose per TBW and per LBW with AUC, the dose per LBW was found to most strongly predict overall systemic exposure. Most notably, approximately 90% of the AUC variability could be accounted for by the folic acid dose per LBW. If the dose per LBW can account for 90% of the systemic exposure variability to folic acid, bioavailability and total apparent clearance would be relatively constant among individuals. This is substantially different from xenobiotics, where total apparent clearance rates can vary greatly, thereby highlighting the tight control the body holds on folate absorption, distribution, and elimination.

Though other groups have not investigated the single-dose pharmacokinetics of folic acid, the relationship between blood concentration response to folic acid administration and LBW has previously been considered. In 2008, Winkels and colleagues hypothesized that, following long-term folic acid administration, red blood cell folate concentrations would be inversely proportional to LBW, on the basis that folate is a hydrophilic molecule and water-soluble drugs are often dosed based on LBW (Winkels *et al*., 2008). Using data from 12-week and 3-year trials of folic acid administration, they found that LBW negatively correlated with red blood cell folate response in both trials. It was estimated that LBW could explain 56-70% of the response to folic acid administration in both trials. Accordingly, our results are in concordance with this finding that, at a fixed folic acid dose, individuals with a higher LBW will have a lower folate response. However, these studies were

conducted in older individuals (50-70 years old), and may not be relevant to younger women of childbearing age.

Other studies comparing the weight-adjusted pharmacokinetics of several antibiotics have been conducted that demonstrate similar results as found in our study. Two groups administered doses of 4 mg/kg of daptomycin to healthy obese and non-obese individuals, and noted significantly higher plasma AUC and C_{max} among the obese participants (Dvorchik and Damphousse, 2005, Pai *et al*., 2007). Dvorchik and colleagues, but not Pai and colleagues, identified a moderately higher CL and V_d in the obese group, and attributed the differences to an elevated LBW as the most reasonable explanation.

Hollenstein and colleagues coordinated a similar study to the previous two; however, pharmacokinetic variables were analyzed beyond plasma AUC (Hollenstein *et al.*, 2001). Obese and non-obese participants were administered 2.85 mg/kg of ciprofloxacin, and plasma AUC and C_{max} were found to be significantly higher in the obese group. Most interestingly, interstitial AUC (for skeletal muscle and subcutaneous adipose tissue) was found to be similar between the groups. While the findings regarding subcutaneous adipose tissue are in concordance with our study results, those regarding skeletal muscle would appear to contradict our conclusions. However, ciprofloxacin displays a substantially higher volume of distribution than folic acid, and it is difficult to extrapolate these findings when the molecular differences are considered. Nonetheless, the authors of this study concluded that reduced perfusion and penetration rates into adipose tissue might explain the pharmacokinetic differences between groups. Our current study did not explore

measures of systemic exposure beyond plasma AUC, and thus cannot rule out tissue perfusion in obesity as possible rationale for our results.

5.2.1. Study limitations

In the current study, BMI was calculated using self-reported weight and height to remain consistent with the earlier study from which data was obtained. Consequently, it is possible that inaccurate self-reported anthropometric measurements may have skewed BMI calculations. A systematic review conducted in 2003 by Engstrom and colleagues found that, in general, self-reported data results in women overestimating height and underestimating weight (Engstrom *et al*., 2003). In all 34 studies examined, women underestimated weight, from a mean low of 0.2 kg to a mean high of 3.54 kg. Moreover, of the studies that examined self-reported accuracy based on actual calculated weight, 95% found that the magnitude of underestimation increased among heavier women. A recent analysis of US data from the 1999-2006 NHANES found that weight is underestimated in women by approximately 1.25 kg, on average (Jain, 2010). Assuming similar trends occurred in our study sample, it is conceivable that LBW was generally underestimated, with the effect being stronger among the obese participants. Further studies should ensure accurate anthropometric measurements are obtained.

The use of a prenatal multivitamin containing folic acid, versus using folic acid alone, may be viewed as another limitation. However, it has been established that the bioavailability of folic acid (as a supplement) is 100%, and other vitamin and mineral components do not hinder absorption. When administered orally with food, folic acid

bioavailability may decrease to approximately 85% (Pfeiffer *et al*., 1997); administering folic acid following a 6-hour fast prevented this issue.

The scoring of prenatal multivitamins to achieve doses calculated to the precision of one-hundredth of a milligram may not be as accurate as administering precisely known quantities of folic acid. Folic acid was assumed to be uniformly distributed throughout the tablet, and pills were shaved to arrive at doses proportional to the remaining weight of the tablet. This methodology was used to remain consistent with administration of the same prenatal multivitamins used in the prior pharmacokinetic studies. However, it is doubtful that such techniques will achieve exact doses equal to those calculated for administration. Nonetheless, it is unlikely that our results indicating a strong and consistent correlation between mg/kg_{LBW} and AUC would be obtained with folic acid doses highly variable from those believed to have been administered.

5.3. Predicted folic acid dose requirements for obese women of childbearing age

Current supplementation guidelines in Canada recommend that all women of childbearing age consume a daily multivitamin containing 0.4 mg of folic acid (Wilson *et al*., 2007), though NTD risks can be further reduced by taking an increased dose (Wald *et al*., 2001). Most available prenatal multivitamins contain between 0.8 and 1.0 mg of folic acid. As neural tube closure occurs early in pregnancy, and often prior to the woman being aware of the pregnancy, guidelines note that all women considering pregnancy should ensure adequate supplementation for at least three months prior to conception.

However, the identification of several risk factors for NTDs has resulted in both the Society of Obstetricians and Gynecologists of Canada and the Motherisk program changing their supplementation guidelines for at risk populations (Wilson *et al*., 2007). These include patients with health risks (e.g. epilepsy, diabetes), a family history of NTDs, belonging to certain ethnic groups, poor medication compliance, lack of birth control, use of certain teratogenic substances, and, most importantly, obesity. For these patients, current guidelines recommend daily supplementation with a multivitamins containing 5.0 mg of folic acid for at least three months prior to, and 12 weeks following, the period of conception.

These recommendations, however, were based on the knowledge that folate deficiency associated with these at risk populations can increase the risk for NTDs. No studies have yet confirmed that an elevated dosage for these individuals can effectively reduce the risk for birth defects. Accordingly, we intended to investigate, based on our

previous findings regarding folic acid and LBW, whether an elevated dosing regimen is justified for obese women of childbearing age. Using the estimated steady-state serum folate concentrations from our data, we determined the dose of folic acid per kilogram LBW that would be expected to maintain serum folate at concentrations of ≥15.9 nmol/L, levels that carried the lowest NTD risk in the study conducted by Daly and colleagues (Daly *et al*., 1995).

As outlined in Table 9, the findings indicate that folic acid dosage requirements (based on LBW) do differ among BMI categories. A relatively linear increase in folic acid intake appears necessary to achieve steady-state levels of ≥15.9 nmol/L as BMI escalates from non-obese to obese values. However, on the whole, it would appear that obese individuals require only a slightly increased dose (approximately 0.3-0.5 mg) as compared to non-obese individuals (approximately 0.2-0.4 mg), since LBW differences are not as extensive as those of TBW across a wide BMI range. Due to dosing being based on LBW, folic acid necessities are more closely tied to differences in height than weight, as increases in height can affect LBW more drastically than weight. For example, a non-obese woman with a weight of 80 kg and height of 1.90 m would require a folic acid dose of 0.38 mg. However, an obese woman with a weight of 110 kg and a height of 1.46 m would only require a dose of 0.34 mg.

Most notably, our results suggest that folate deficiency may not explain the increased NTD risk among obese individuals. According to our findings, certain individuals at even the upper extremes of obesity would require a dose of approximately 0.4 mg to

reach protective folate concentrations. As previously mentioned, studies that identified an increased risk for NTD-affected pregnancies among obese mothers found the risk remained even after controlling for adequate supplementation with folic acid. As general supplementation guidelines have always recommended a folic acid dose of *at least* 0.4 mg (often up to 1.0 mg), our data would suggest that these individuals were likely supplementing at sufficient levels. Consequently, these results demonstrate that periconceptional guidelines already recommend adequate folic acid supplementation to obese women of childbearing age, when considering pharmacokinetic variabilities.

Our findings are, however, based on the assumption that achieving serum folate concentrations of ≥15.9 nmol/L will lead to the greatest level of NTD protection among obese women of reproductive age. This premise may not necessarily be accurate. The initial study data upon which Daly and colleagues arrived at their conclusions regarding blood folate status and NTD risk was collected from a population of pregnant women in Ireland between 1986 and 1990 (Kirke *et al*., 1993). Though participant information regarding weight or BMI was not provided, there is little reason to believe that the study was conducted within a largely obese sample. Thus, it may be unsuitable to generalize these results to obese women. Additionally, the Daly study was unable to correlate further increases in folate status with the risk for NTDs. It is possible that increases in serum folate concentrations beyond 15.9 nmol/L will lead to a further reduction in NTD risk. This possibility, if true, would justify elevating the dosage guidelines not only for obese individuals, but for non-obese as well.

Nonetheless, our results may serve to reduce anxiety and increase compliance among obese individuals whom were previously apprehensive about consuming higher doses of folic acid. Concerns regarding elevated folic acid intake became apparent following nation-wide fortification of grain products. Studies identifying higher-than-expected folate intake due to fortification (Quinlivan and Gregory, 2003) raised concerns about circulating blood levels of unmetabolized folic acid (Kelly *et al*., 1997) and an increased risk for colorectal cancer (Kim, 2007). As cancer cells require an abundance of folate to proliferate, higher folic acid intake has been theorized to increase the risk for those predisposed to certain cancers and those with existing neoplasms (Smith *et al*., 2008). Though a recent randomized control trial (Jaszewski *et al*., 2008) and meta-analysis (Kennedy *et al*., 2011) were unable to link high folic acid consumption with an increased risk for colorectal cancer, our results may nonetheless reassure otherwise hesitant obese individuals that non-supratherapeutic doses are sufficient to protect against NTDs.

5.3.1. Should current guidelines for folic acid supplementation be changed?

In summary, our results suggest that the association between obesity and NTD risk may not be related to insufficient folate supplementation in obese women. However, it is important to note that present trends regarding supplementation rates among women of childbearing age warn against altering guidelines based on this data. A Canadian Community Health Survey in 2005 found that only 57.7% of women of childbearing age supplement with folic acid for at least three months prior to pregnancy (Public Health Agency of Canada, 2008). Additionally, a recent US study indicated that obese women of childbearing age are 24% less likely to supplement with folic acid daily (Case *et al*., 2007).

Daily supplementation rates were found to be substantially lower even after controlling for both recommendations from a healthcare provider and having knowledge that folic acid prevents birth defects (OR 0.65, 95% CI 0.49-0.87). As adherence appears to be reduced in the obese population, higher doses of folic acid may be necessary to ensure a protective folate status is maintained despite missed doses.

Furthermore, with 39-57% of pregnancies in Canada and the US being unplanned (Forrest, 1994), higher doses may be required to more rapidly achieve a protective folate status prior to neural tube closure (Nguyen *et al*., 2009). Even among planned pregnancies, adequate folic acid supplementation may not be achieved by neural tube closure, as it occurs at approximately 4 weeks post-conception, a point at which many women may be unaware of their pregnancy. Therefore, with both compliance and unplanned pregnancy enduring as prevalent issues, bringing the current folic acid supplementation guidelines for obese individuals in line with those for non-obese populations may be a premature and unwarranted initiative.

5.3.2. Study limitations

It remains possible that the increased risk for NTDs among obese mothers is related to elevations in LBW, which are more pronounced for taller individuals classified as obese. As can be noted in Table 9, folic acid doses approaching (and above) 0.5 mg would be required for obese women ≥1.80 m in height. Furthermore, results obtained by Werler and colleagues appeared to indicate a non-significant trend towards an elevated NTD risk among individuals ≥1.70 m in height (RR 1.3, 95% CI 0.7-2.6) (Werler *et al*., 1996). However,

these results are unable to account for the fact that the NTD risk was consistently found to escalate with a rise in BMI, with morbidly obese individuals (most commonly found among those shorter in stature) being at the highest risk.

Our study does present with several other limitations. First, steady-state serum folate concentrations were estimated from single-dose pharmacokinetic data. Actual steady-state data should be established prior to changing current supplementation guidelines. Nonetheless, previous pharmacokinetic studies have estimated steady-state plasma concentrations from single-dose data with relatively accurate precision (Alexanderson, 1973). Second, while our data would indicate that current folic acid supplementation recommendations during the periconceptional period are sufficient for obese individuals, it is conceivable that a functional deficiency exists. Obese women have a higher incidence of gestational diabetes, insulin resistance, elevated testosterone levels, and other metabolic disorders (Mokdad *et al*., 2003, Kissebah and Peiris, 1989), each which may lead to an increased risk for NTDs via unrecognized folate interactions. It is still possible, however, that supratherapeutic levels of folic acid may attenuate these risks.

As described earlier, the baseline serum folate status of the sample used was relatively consistent with that of other population-based studies. There is reason to believe, however, that the estimated folic acid doses may not apply to those with a lower or deficient serum folate status. Previous work has found that, at a fixed folic acid dose, those with a lower baseline folate status will display a greater serum response following long-term administration as compared to those with a higher baseline status (Rosenthal *et al*.,

2008). Following folic acid administration of 1.0 mg/day for 12 weeks, participants in the lowest baseline serum folate quartile increased their folate status by 230%, while those in the highest quartile only increased it by 81%. Regardless, these findings would not affect our study conclusions; taking them into account would only serve to reduce the estimated folic acid dose required for obese individuals to reach and maintain protective serum folate concentrations.

CHAPTER 6. CONCLUSIONS & FUTURE DIRECTIONS

Aside from folate deficiency, there is a paucity of literature explaining the mediating causative mechanisms between several risk factors and NTDs. Obesity, originally recognized as a risk factor for NTD-affected births in 1994, is no different. Early studies investigating the association between maternal weight and pregnancy outcome consistently identified elevated BMI as a risk factor. Unfortunately, controlling for a variety of factors, including adequate folic acid intake and obesity-associated comorbidities, did not reveal any confounders as responsible for the increased risk. More recent studies continue to establish obesity as a risk factor for NTDs; yet, causative mechanisms remain unresolved.

While many of these studies did control for adequate folic acid intake, blood folate status was not examined to ensure that protective folate concentrations were reached. Population-wide blood vitamin level investigations have found that obese individuals tend to have a decreased serum folate status, even when folic acid intake is similar to non-obese individuals. Accordingly, we hypothesized that folic acid pharmacokinetic parameters may be altered in the obese population, thus necessitating a different dosage than currently recommended for all women of childbearing age. To evaluate this, pharmacokinetic parameters were assessed following administration of a weight-adjusted folic acid dose in obese and non-obese participants. To the best of our knowledge, folic acid pharmacokinetics have not been previously compared between obese and non-obese women of childbearing age.

Analysis of pharmacokinetic parameters found that overall systemic exposure, as represented by AUC, was elevated in the obese group. Other parameters, such as $t_{1/2}$, V_d, and CL, did not exhibit any significant differences. While the absolute dose administered to the obese group was higher, the AUC should theoretically have been similar between groups, as the folic acid would distribute into the larger body, resulting in relatively equivalent plasma concentrations. The higher AUC, however, indicated that folic acid was not freely distributing into the adipose body. Other measures of body composition were considered to explain these differences.

LBW, an anthropometric measure that is moderately elevated in obese individuals, and is often employed for calculating weight-adjusted dosage for hydrophilic drugs, was found to account for the AUC differences between groups. AUC was more strongly predicted after normalizing the dose to kilogram LBW, as opposed to kilogram TBW. Remarkably, 90% of the AUC variability was explained by the dose per kilogram LBW, indicating the tight control the body holds over systemic folate concentrations.

Using these data, we estimated the daily folic acid dose (based on LBW) that would achieve serum folate levels of ≥15.9 nmol/L for women at a wide range of BMI values. While daily doses were, on average, slightly higher for obese individuals as compared to non-obese individuals, most individuals would achieve this folate status with a dose of approximately 0.4 mg. Therefore, this would indicate that current supplementation guidelines do in fact recommend an adequate amount of folic acid to protect against NTDs.

In conclusion, the current study did not identify alterations in folic acid pharmacokinetics among obese women of childbearing age, nor did it estimate that dosage recommendations should be elevated for this population. Supplementation guidelines recommend women of childbearing age to consume 0.4 mg of folic acid daily, and most prenatal vitamins contain between 0.6 and 1.0 mg. As a result, even individuals at the upper extremes of BMI values are consuming sufficient amounts of folic acid. Nonetheless, altering guidelines based on this data may be a premature initiative. Compliance and unplanned pregnancy continue to be concerns, and higher folic acid doses may be required to avoid low folate status as a consequence of these issues. Additionally, this study is unable to rule out a functional deficiency associated with obesity in pregnancy. Further studies must be conducted to fully comprehend the link between obesity and NTD-affected births.

6.1. Future Directions

While our study was able to identify how the folic acid dose per kilogram LBW determines overall systemic exposure, our sample population and study methodology preclude us from asserting that folate deficiency does not mediate the increased NTD among obese pregnancies. Further investigations would be able to more thoroughly examine this issue.

6.1.1. Additional folic acid pharmacokinetic investigations

Our current study recruited a sample of non-pregnant women of childbearing age; however, several other study methodologies may reveal pharmacokinetic differences between obese and non-obese individuals that ours was unable to uncover. Conducting a similar study over a period of several months to examine steady-state folate concentrations may reveal long-term differences in systemic exposure that cannot be accounted for by the folic acid dose per kilogram LBW, or that cannot be accurately estimated from single-dose data.

In addition, utilizing a sample population of obese and non-obese pregnant women could potentially reveal pregnancy-associated variations in folate exposure. As neural tube closure occurs around 4 weeks gestation, these studies would be most accurate if conducted at or prior to this point. Both single-dose (at approximately 4-6 weeks gestation) and steady-state (leading up to 6 weeks gestation) studies could unveil alterations in early pregnancy that may necessitate dosing adjustments in the obese population. Though plasma volume expansion is minimal early in pregnancy (Hytten and Paintin, 1963), other

adaptations could theoretically change the folic acid dose required to sustain protective serum concentrations.

6.1.2. Retrospective study of folic acid use among obese mothers

Though our estimates suggest that current guidelines recommend an adequate dose of folic acid for women across a wide range of BMI values, it would be interesting to note whether increased doses confer a higher level of protection against NTDs among obese women of childbearing age. This could be conducted by surveying a population of obese women, including both those with healthy births and those whom were affected by NTDs, and comparing folic acid intake among the groups. Several groups have suggested guidelines be altered to recommend doses of up to 5.0 mg (Wald *et al*., 2001, Wilson *et al*., 2007); thus, it is possible that sufficient women could be recruited to detect the effect of different doses of folic acid on pregnancy outcome. This study would provide the most direct and applicable evidence for folic acid dose requirements among obese women of childbearing age.

6.1.3. Association between folate status and NTD risk in obese mothers

Daly and colleagues landmark study investigating the association between folate status and NTD risk was conducted in an Irish population, which likely did not contain a large proportion of obese individuals (Daly *et al*., 1995). Thus, it is difficult to extrapolate their findings to an obese population. Discovering whether a similar association exists (at similar folate statuses) in obese women would be imperative to substantiating our findings.

Such a study would be able to investigate whether a further increase in folate status would bring a further reduced incidence of NTDs.

6.1.4. Consideration of functional folate deficiency

As previously mentioned, achieving and maintaining serum folate concentrations ≥15.9 nmol/L in obese individuals would not necessarily eliminate a folate-associated risk for NTDs, as these concentrations were not originally defined among a population of obese women. Indeed, a functional folate deficiency in obese pregnancies may exist, whereby certain folate concentrations are achieved, though circulating folates are not directed towards the targeted compartments (i.e. fetus). Studies have linked non-alcoholic fatty liver disease, a condition estimated to affect 57-74% of obese individuals (Angulo, 2002), with lower circulating serum folate concentrations (Hirsch *et al*., 2005). As circulating folate may be directed towards hepatic compartments for tissue repair, fetal exposure to active folate species may decrease. Future studies should consider this potential functional deficiency, as well as others, when investigating the elevated NTD risk associated with obesity. Additionally, it would be of particular importance to examine the association between serum folate concentrations and NTD risk among obese women of childbearing age.

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LIST OF PUBLICATIONS & ABSTRACTS

PUBLICATIONS

Stern SJ, Matok I, Kapur B, Koren G. A comparison of folic acid pharmacokinetics in obese and non-obese women of childbearing age. *Ther Drug Monit* 2011; 33(3): 336-40.

Kennedy DA, **Stern SJ**, Matok I, Sarkar M, Nickel C, Koren G. Folate intake and the incidence of colorectal cancer: a systematic review and meta-analysis. *Cancer Epidemiol* 2011; 35: 2-10.

ABSTRACTS

Stern SJ, Matok I, Kapur B, Koren G. A comparison of folic acid pharmacokinetics in obese and non-obese women of childbearing age. *J Popul Ther Clin Pharmacol* 2011; 18(2): e323-4.

Stern SJ, Matok I, Kapur B, Koren G. A comparison of folic acid pharmacokinetics in obese and non-obese women of childbearing age. *Clin Pharm Ther* 2011; 89(S1): S94.

Kennedy DA, **Stern SJ**, Matok I, Sarkar M, Nickel C, Koren G. Folate intake and the incidence of colorectal cancer: a systematic review and meta-analysis. *Clin Pharm Ther* 2011; 89(S1): S49.

Stern SJ, Koren G. Understanding the relationship between obesity and folate status in the protection against neural tube defects: a systematic review. *Birth Def Res Part A* 2010; 88(5): 434.

APPENDICES

SickKids®
THE HOSPITAL FOR SICK CHILDREN
Research Ethics Board

Research Consent Form

Title of Research Project:

Optimizing Periconceptional and Prenatal Folic Acid Supplementation

Investigator(s):

Principle Investigator: Dr. Gideon Koren, MD
Director of Motherisk Program
The Hospital for Sick Children
Telephone: 416-813-7283

Purpose of the Research:

We wish to measure serum folate concentrations among women of childbearing age before and after a single dose ingestion of a folic acid supplement. We want to compare folate blood measurements among women of various weight ranges, in both 1.1 mg folic acid (PregVit®) and 5.0 mg folic acid (PregVit-Folic 5®) equivalent groups. The results of this study may be important in determining the optimal dose of folic acid for planning and pregnant women to take in order to reduce the risk for neural tube defects.

Description of the Research:

This is a *randomized study*, which means that a randomization process was used to assign, by chance alone, which group each participant belongs to. Thus, you were assigned by chance to the ☐ PregVit-Folic 5® / ☐ PregVit® group. The actual dose you will receive is dependent upon your weight, and will be told to you at the beginning of the study. The two groups are in *equipoise*, which means they are considered equal and it is unknown which arm is better. All vitamins and mineral doses, aside from folic acid, are identical between the two supplements.

The following flow chart outlines the steps of participation:

Research coordinator and potential participant discuss the study.
⇩
Written consent.
⇩
Enrolment into study and randomization.
⇩
Participant comes to research site after a 6 hour fast.

⇩

5 mL blood sample (via indwelling catheter) will be drawn
before multivitamin supplementation is initiated.

⇩

Ingest folic acid dose.

⇩

Blood samples will be drawn 8 times over the course of 10 hours after dose administration.
(Sampling times: 0.5, 1, 2, 3, 4, 6, 8, and 10 hours)

⇩

4 hours after ingestion of folic acid dose, a meal will be offered.

⇩

Telephone interview will be conducted to document diet.

The total volume of blood that will be taken is about 50 mL (10 teaspoons). The day-long appointment will be scheduled according to when *you* are available.

Potential Harms:

High doses of folic acid can mask vitamin B12 deficiency. However, this is generally not a concern for healthy individuals, with no chronic medical conditions. One study has shown that vitamin B12 deficiency can still be detected even with high folate blood concentrations.

Potential Discomforts or Inconvenience:

The one-time needle poking may not be pleasant. We will offer you a cream named EMLA® to massage on your arm, which takes away much (sometimes all) of the pain of poking. An alternative that can be used is a gel named Ametop®.

Potential Benefits:

We will be able to tell you your folate blood levels. Results can be disclosed in person or mailed. Daily multivitamin supplementation can improve vitamin and mineral concentrations.

☐ I would like to know the results of the folate blood measurements.

☐ In person ☐ By mail

Alternatives to participation:

You are asked to volunteer for this study. There are no consequences if you do not participate.

Confidentiality:

We will respect your privacy. No information about who you are will be given to anyone or be published without your permission, unless the law requires us to do this. For example, the law requires us to give information about you if a child has been abused, if you have an illness that could spread to others, or if the court orders us to give them the study papers"

Sick Kids Clinical Research Monitors, employees of the funder or sponsor of the study, or the regulator of the study may see your health record to check on the study. For example, people from Health Canada Health Products and Food Branch, U.S. National Institutes of Health, or U.S. Food and Drug Administration, if necessary, may look at your records."

By signing this consent form, you agree to let these people look at your records. We will put a copy of this research consent form in your patient health records. We will give you a copy for your files.

The data produced from this study will be stored in a secure, locked location. Only members of the research team (and maybe those individuals described above) will have access to the data. This could include external research team members. Following completion of the research study, the data will be kept as long as required and then destroyed as required by Sick Kids policy. Published study results will not reveal your identity.

Reimbursement:

We will reimburse you for your participation at $200 upon your completion of the study protocol. Under certain circumstances (as assessed by the study's medical advisors), we will pro-rate payments depending on the degree of participation.

Participation:

Participation in research is voluntary. If you choose not to participate, you and your family will continue to have access to quality care at the Hospital for Sick Children. If you choose to participate in this study you can withdraw from the study at any time. Your participation may contribute to the creation of new diagnostic tests, new medicines or other events that may have commercial value. However, your participation in this study will not entitle you to a share in any future economic benefit.

New information that we get while we are doing this study may affect your decision to take part in this study. If this happens, we will tell you about this new information. And we will ask you again if you still want to be in the study.

During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you any of this money now or in the future because you took part in this study".

If you become ill or are harmed because of study participation, we will treat you for free. Your signing this consent form does not interfere with your legal rights in any way. The study staff, any people who gave money for the study, or the hospital are still responsible, legally and professionally, for what they do.

Sponsorship:

The sponsor of this research is Duchesnay Inc. (Laval, Quebec).

Conflict of Interest:

Duchesnay Inc. supports the *Nausea and Vomiting of Pregnancy (NVP) Healthline* at the Motherisk program, and Dr. Koren is a medical consultant for Duchesnay Inc.

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"By signing this form, I agree that:
1) You have explained this study to me. You have answered all my questions.
2) You have explained the possible harms and benefits (if any) of this study.
3) I know what I could do instead of taking part in this study. I understand that I have the right not to take part in the study and the right to stop at any time. My decision about taking part in the study will not affect my health care at Sick Kids.
4) I am free now, and in the future, to ask questions about the study.
5) I have been told that my medical records will be kept private except as described to me.
6) I understand that no information about who I am will be given to anyone or be published without first asking my permission.
7) I have read and understood pages 1 to 5 of this consent form. I agree, or consent, to take part in this study.

_____	_____
Printed Name of Subject & Age	Subject's signature & date
_____	_____
Printed Name of person who explained consent	Signature & date
_____	_____
Printed Witness' name (if the subject/legal guardian does not read English)	Witness' signature & date

If you have any questions about this study, please call Seth Stern at 416-813-7283.

If you have questions about your rights as a subject in a study or for information on whom to contact in the event of injuries during a study, please call the Research Ethics Manager at 416-813-5718.

Enrolment Intake Form

MOTHERISK TREATING THE MOTHER – PROTECTING THE UNBORN
SickKids

Patient No. _____	**Study Participation:**
Consultation Date: _____ **Study Co-ordinator:** _____ Seth Stern	Part 4. Measuring and Comparing Serum Folate Levels Before and After Single-Dose Administration of Folic Acid Among BMI Groups ☐ PregVit® Supplementation Group ☐ PregVit-Folic 5® Supplementation Group Part 5. Measuring and Comparing Serum and Red Blood Cell Folate Levels in Non-Pregnant Women Among BMI Groups ☐ PregVit® Supplementation Group ☐ PregVit-Folic 5® Supplementation Group

Patient Information

Patient Name _____

Home Phone _____ Work/Cell Phone _____

Address _____

City _____ Province _____ Postal Code _____

E-mail (optional) _____

Date of Birth _____

Weight _____ lbs / kg Height _____ ft / m

LMP _____ Is it regular? ☐ No ☐ Yes _____ days

G _____ P _____ SA _____ TA _____ ectopic _____ molar _____

Appendix B: Enrollment Form

Demographics

Maternal ethnicity	Marital status	Highest education	Employment
☐ Caucasian ☐ Black ☐ Hispanic ☐ Oriental Asian ☐ Indo-Asian ☐ Other _____	☐ Single ☐ Married ☐ Common Law ☐ Divorced ☐ Widowed	☐ Public School ☐ High School ☐ College ☐ University ☐ Post-Graduate	☐ Unemployed ☐ Homemaker ☐ Student ☐ Employed ☐ FT ☐ PT ☐ Self-Employed ☐ FT ☐ PT

Medications and Exposures

☐ None

Drug	Indication	Start	Stop	Dose/Route	Side Effects

Substance Use

☐ None

Substance	Start	Stop	Dose/Frequency
☐ Alcohol			
☐ Tobacco/Nicotine			
☐ Marijuana			
☐ Other _____			

Medical History

☐ No medical conditions, healthy overall

Category	Condition	Notes
Thyroid	☐ Hypothyroidism ☐ Hyperthyroidism ☐ Other _____	_____ _____ _____
GI	☐ Crohn's disease ☐ Ulcerative colitis ☐ Peptic/duodenal ulcer ☐ Irritable colon ☐ IBS ☐ GERD ☐ NVP ☐ Heartburn, reflux ☐ Indigestion ☐ Abdominal discomfort ☐ Constipation ☐ Diarrhea ☐ Other _____	_____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____ _____
Organ	☐ Heart disease ☐ Liver disease ☐ Kidney disease ☐ Other _____	_____ _____ _____ _____
Miscellaneous	☐ Hypertension ☐ Diabetes ☐ Migraines ☐ Anemia ☐ Bacterial/viral infection ☐ Asthma ☐ Other _____	_____ _____ _____ _____ _____ _____ _____
Psychiatric	☐ Anxiety ☐ Depression ☐ Bipolar ☐ Other _____	_____ _____ _____ _____
Other		_____ _____ _____ _____