Review

Obesity, neural tube defects and folic acid---A complex relationship

Gideon Koren[1,*], Yusuf C Kaplan[2,3]

[1] Head of Pharmacology, Adelson school of medicine, Ariel University, and Director, Motherisk International, 40700 Ariel, Israel

[2] Terafar-Izmir Katip Celebi University Teratology Information, Training and Research Center, 35620 Izmir, Turkey

[3] Izmir University of Economics Faculty of Medicine, 35330 Izmir, Turkey

*Correspondence: gidiup_2000@yahoo.com (Gideon Koren)

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Obesity is associated with twofold increased risk of neural tube defects (NTD). Research has repeatedly shown that about 70% of NTD are folic-acid dependent. Yet, there is controversy whether folic acid status is the main determinant of the increased risk of obesity-induced NTD. The rational for this review is to update and discuss the evidence on the link between obesity, folic acid and NTD, in an attempt to shed light on the question whether optimal folic acid dose schedule can mitigate this risk. During pregnancy maternal folate requirements increase by 5--10-fold, as folate is diverted towards the placenta and fetus, as well as supporting different maternal organs. Correspondingly, low maternal folate status has been associated with birth defects in fetal anatomical regions particularly sensitive to reduced folate intake including oral cleft, cardiovascular defects and NTD. A recent study has documented decreased placental folate transporter expression and activity in the first and second trimesters among obese mothers. This may explain the higher incidence on NTD in infants of obese women, as less folate may find its way to the developing fetus during the sensitive periods for creating NTD. Recent pharmacokinetic results indicate that steady state levels of folate are almost perfectly defined by the dose per lean body weight (LBW). The mean dose per kg LBW that would be expected to result in steady state serum folate level of $>$ 15.9 nmol/L was identified as 0.0073 mg/kg LBW. A large study found no differences in dietary supplementations of folic acid, yet obese women exhibited lower median serum folate as well as lower mean serum B12 levels, but no differences in mean RBC folate levels. There was a negative correlation between increasing BMI and both serum folate and plasma B12. Future research will be needed to incorporate more fully, in addition to evidence of NTD, obesity and folic acid intake, also direct measurements of serum and RBC folate, as well as other confounders, in order to create a model that will shed light on these complex interactions.

Keywords

Obesity; Pregnancy; Folic acid; Neural tube defects; Spina bifida; Congenital abnormalities

1. Introduction

Obesity is associated with twofold increased risk of neural tube defects (NTD) [1]. Research has repeatedly shown that about 70% of NTD are folic-acid dependent. Yet, there is controversy whether folic acid status is the main determinant of the increased risk of obesity-induced NTD.

It is surprising that very little research has been published on folic acid dose optimization for obese women planning pregnancy. This question is highly relevant to millions of women worldwide, and measurements of folic acid are abundantly available. With numerous health organizations maintaining electronic health records, it should not be difficult to better characterize the complex relationship between folic acid dose, folate serum and RBC levels, BMI and NTD.

Studies have shown that only a minority of overweight and obese women achieve the recommended folic acid supplementation of 400 mcg/d [2]. Further studies discussed here have shown that, even after adjusting for folic acid consumption of 400 mcg/d, the risk of NTD remains elevated. However these studies did not examine the resultant folate circulating levels and hence, did not address potential changes in the pharmacokinetics of folate in obesity.

The objective of this review was to update and discuss the evidence on the link between obesity, folic acid and NTD, in an attempt to shed light on the question whether optimal folic acid dose schedule can mitigate this risk.

2. Methods

We conducted a scoping review of studies investigating obesity in pregnancy, neural tube defects, obesity and folate status. The focus was the complex relationship between obesity, NTD and folate status. For that end we searched PubMed, Medline, Embase, Chochrane, Google and Goole Scholar from inception to 01 July 2020.

2.1 Obesity and pregnancy

Maternal obesity during the peri-conceptional period is a growing public health issue, as the prevalence of obesity in the general population rises to unprecedented highs. In this Special Issue a variety of pregnancy-related complications associated with obese mother are delineated.

Maternal obesity, is defined by body mass index (BMI) greater than or equal to 30 kg/m^2. In Canada, for example, among non-pregnant women between the ages of 15–55 years old, 21.1% are defined overweight (BMI 25.0–29.9 kg/m^2), and 12.1% as obese. In the USA the prevalence of obesity is even higher, with 28.9% are obese and 8.0% approaching levels of morbid obesity ($>$ 40 kg/m^2) [3].

Obesity is associated with a long list of risks for the expecting mother and her offspring, including miscarriage, stillbirth, malformations, gestational diabetes, preeclampsia, macrosomia, need for Cesarian section and impaired growth [4].

2.2 Neural tube defects

NTD are a class of congenital malformations characterized by failure of the neural tube to close during embryogenesis. The neural tube, a precursor for the central and peripheral nervous systems is typically fully developed and fused by 21–28 days post-conception [5]. The main forms of NTD are spina bifida (incomplete closure at the caudal end), anencephaly (incomplete closure at the cranial end), and there are also other, less common forms, including craniorachischisis, encephalocele and iniecephaly.

Children born with NTD may display a wide range of disabilities, from early death (with anencephaly), open spinal sac (in spina bifida) with various degrees of permanent neurological damage [6], involving various degrees of physical disability and paralysis, reduced IQ and impaired psychological development [7]. Mean direct lifetime cost per infant with spina bifida is estimated to be $791,900, or $577,000 excluding caregiving costs [8].

Presently, NTD are the second most prevalent group of major congenital malformations after cardiovascular anomalies [7–9]. Following the discovery of folic acid prevention of NTD, and the implementation of flour fortification and food supplementation, the prevalence of NTD has steadily decreased in countries that follow directives for fortification and supplementation. As an example, in Canada, following fortification of grain products with folic acid, NTD prevalence rates fell from 1.58 per 1000 births to 0.86 per 1000 [10].

In addition to folate deficiency, several other risk factors for NTD have been suggested, including exposure to organic solvents, arsenic, pesticides, and drugs such as antifolate medications (sulfonamides, valproic acid) and dihydrogesterone [11].

2.3 Folic acid

Folic acid, or pteroylmonoglutamic acid, refers to the oxidized form of folate, the water soluble B group vitamin. Folates are synthesized abundantly in green, leafy vegetables, various legumes and moderately in certain fruit juices and animal liver store high amounts of this vitamin. Humans, similar to other mammalians, cannot synthesize folate, and it must be consumed to maintain adequate levels. Folic acid, the fully oxidized form, allows greater stability, is less susceptible to degradation, and hence it is used for supplementation and food fortification [12].

2.3.1 Folate pharmacokinetics

Folic acid bioavailability differs from that of natural folates, with synthetic folic acid often approaching nearly 100%, as compared to 50% for natural dietary folates [13]. When consumed as part of fortified food products, rather than as supplement, the bioavailability is around 85%. The different bioavailability stems from the length of the polyglutamate chains and stability of the oxidized folate. This reality has led to the establishment of Dietary Folate Equivalents (DFE) to be able to compare the different forms of folate intake [14]. For example, folic acid consumed alongside a meal or as part of fortified product, is estimated to be 1.7 fold more bioavailable than dietary folate.

While folic acid is absorbed unchanged, natural folates must first be hydrolyzed to remove the polyglutamate chains by the enzyme glutamate carboxypeptidase II. During transport across the intestine, the enzyme dihydrofolate reductase (DHFR) reduces folate to dihydrofolate (DHF). At low levels of folate intake, most elimination takes place through biliary secretion. At serum levels above 45 nmol/L, significant amounts are lost in the feces and urine. Once incorporated into red blood cells, the elimination of RBC folate has a half-life of 8 weeks. Folate plays a critical role in cell growth and replication and is involved in numerous major reactions, including the synthesis of thymidylate, purines, methionine, catabolism of histidine and the conversion of serine to glycine [15].

2.3.2 Folic acid and pregnancy

During pregnancy maternal folate requirements increase by 5–10 fold, as folate is diverted towards the placenta and fetus, as well as supporting different maternal organs. Correspondingly, low maternal folate status has been associated with birth defects in fetal anatomical regions particularly sensitive to reduced folate intake, including oral cleft, cardiovascular defects and NTD [16]. Evidence accumulating since the 1980 has established that folate deficiency is associated with tangible risk for NTD, leading to directives for folic acid food fortification and supplementation in most countries worldwide.

In 1995, Daly *et al.* [17] established the correlation between serum and RBC folate levels and the risk of NTD, and these have become the compass for dose recommendations. In general, serum folate levels $>$ 15.9 nmol/L and RBC levels above 906 nmol/L guarantee full protection against folate-dependent NTD, whereas serum levels between 0–4.4 nmol/L are associated with 4 fold higher risk.

Folic acid fortification of grain products has rapidly proven to be a successful public health initiative in increasing numbers of countries. NTD have a multifactorial origin, and presently only 70% of NTD are folic-acid dependent. Hence, the relationship between folic acid levels and NTD is diluted by the 30% cases not dependent on folic acid.

2.4 Obesity and NTD

By 2020 a large number of studies and meta analyses have documented a clear and robust association between obesity and NTD [18]. In a recent large study from China with 194,844 women, there was an over fivefold increased risk of spina bifida among obese women. Among women who took folic acid supplement, the adjusted risk for spina bifida among obese women was tenfold higher than among normal weight women [19]. Studies have attempted to control for potential

confounders, including maternal age, gravidity, education, use of alcohol and cigarettes, chronic illness and folic acid supplementation. Overall, these studies confirmed a nearly twofold increased risk, with a three-fold elevated risk among those at the extreme end of obesity. Existing meta-analyses commonly cited potential mechanisms for these findings, including metabolic disorders and nutritional deficiencies [20].

2.5 Obesity and folate status

As many of the malformations that arise from folate deficiency are also associated with obesity (e.g., NTD, orofacial defects and cardiovascular anomalies), it is conceivable to investigate whether obesity is linked through lower folate status. Indeed, poor nutritional intake is frequently found among obese persons. Consumption of fruits and vegetables is substantially lower among obese [20] and elevated pre-pregnancy BMI correlates negatively with diet quality. De Jersey *et al.* [2] have shown that only 11% of overweight and obese women achieve the recommended folic acid supplementation of 400 mcg/d. Further studies have shown that, even after adjusting for folic acid consumption of 400 mcg/d, the risk of NTD remains elevated [21–23]. However these studies did not examine the resultant folate circulating levels and hence, did not address potential changes in the pharmacokinetics of folate in obesity. Mojtabai *et al.* documented, based on large numbers of subjects, that elevated BMI among non-pregnant women was associated with lower serum folate concentrations both prior to, and following flour fortification with folic acid. When adjusting for overall folate intake, every 10 mg/m^2 increase in BMI led to significant decrease in serum folate [24]. Ortega *et al.* have shown that obese persons were 6-fold more likely to have folate concentrations below 14.9 nmol/L. Obese persons who lost weight showed increased folate levels while overall intake remained unchanged [25].

Compared to women with normal BMI, obese women had lower medium serum folate, but no differences in mean RBC folate [26]. In a recent large American study, overweight was not associated with RBC folate concentrations indicative of NTD risk ($<$ -748 nmol/L), whereas obesity had a marginally increased risk for such levels. In contrast, there was a significant protective association between serum folate levels indicative of NTD risk and BMI above 40 kg/m^2 [27]. Confounders that must be addressed in these analyses stem from high rates of diabetes mellitus, which is a risk factor for NTD, among obese people. Two studies investigating obesity and the risk of NTD have adjusted for diabetes mellitus, and the risk of obesity remained significant [21, 22].

In a recent study Kerr *et al.* [28] have shown an association between maternal periconceptional fever and the risk of NTD and provided evidence that this association was attenuated by folic acid consumption at the recommended levels. Another potential source of confounding lies in the fact that maternal obesity decrease the effectiveness of ultrasound to recognize NTD. However, a meta-analysis noted no change in the elevated risk for NTD among obese women when also pregnancy terminations were counted [29].

Several genetic polymorphisms in the MTHFR enzymes have been associated with increased risk for NTD [30], however, several large population studies have failed to show an association between BMI and 677C-T polymorphism.

A recent study has documented decreased placental folate transporter expression and activity in the first and second trimesters among obese mothers. The authors hypothesized that this may explain the higher incidence on NTD in infants of obese women, as less folate may find its way to the developing fetus during the sensitive periods for formation of NTD [31].

Folate pharmacokinetics in obese women

In a study by Stern *et al.* [32], the pharmacokinetics of a single dose folic acid were compared between 12 obese and 12 non obese, non-pregnant women. The participants received weight-adjusted doses of folic acid. After a minimum of 6 hour fast, the folic acid preparation was administered orally, and blood samples were obtained from a venous line at 0.5, 1, 2, 3, 4, 6, 8 and 10 hours. In addition to body weight and BMI, the authors calculated lean body weight (LBW) by the equation:

LBW (kg) = 9270xKg TBW/8780 + (244x BMI) [33].

No differences in baseline serum folate were detected between the groups. The primary endpoint of the study was the area under the concentration-time curve (AUC). The AUC, (981.2 nmol/h/L vs. 1482.3 nmol/h/L) and peak folate levels (447.4 nmol/L vs. 301.3 nmol/L) were significantly larger among obese women. No differences were detected in half lives, distribution volumes or clearance rates. To explain the larger AUC values among the obese women, lean body weight (LBW) was investigated. When calculating the ratio of LBW/TBW, it became apparent that proportionally, obese women have lower LBW for a given TBW. Because all women (obese and non-obese) received similar dose per TBW (0.035 mg/kg), in essence obese women received significantly larger relative dose per LBW.

Indeed, the correlation between AUC and TBW or LBW, was substantially better for LBW (R2 = 0.90) than for TBW (R2 = 0.76).

Critically, mean steady state concentration of any pharmaceutical is defined by the clearance rate, and NOT by its distribution volume according to the formula:

Steady State Concentration = Dose rate/Clearance rate.

Because the clearance rate of folate was equal between obese (90.7 mL/min) and non-obese (87.9 mL/min) subjects, the mean serum concentration should not differ based on obesity.

These results indicate that steady state levels of folate are almost perfectly defined by the dose (mg/kg LBW). Using this regression line, the mean dose per kg LBW that would be expected to result in steady state serum folate level 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 range of BMI values, a chart is presented with various weights, 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.

Tinker *et al.* have shown, using the National Health and Nutrition Examination Survey, that BMI was inversely associated with serum folate levels among women who did not use supplements containing folic acid. In contrast, no differences between women in different BMI categories were observed among supplement users [34]. Regardless of supplement use, obese women had the highest RBC folate concentrations. These results do not support a straightforward modification of the relationship between folic dose and folate levels according to BMI. The authors hypothesized that BMI may affect folate distribution, with lower serum, but higher RBC concentrations.

DA Silva and colleagues conducted an intervention study to compare the relationship between BMI and short-term pharmacokinetics of folate [35]. Healthy obese and normal -weight women of childbearing age were administered a single dose of 400 mcg of folic acid. Fasting baseline serum folate levels were lower among obese women, while RBC folate levels were higher. Overall AUC was lower among obese women, suggesting that folate distribution is different in obesity, and should pregnancy occur, many reduce the availability of fetal folate.

O'Malley and colleagues studied 492 women and found no differences in dietary supplementations of folic acid. Despite this, obese women exhibited lower median serum folate as well as lower mean serum B12 levels, but with no differences in mean RBC folate levels. There was a negative correlation between increasing BMI and both serum folate and plasma B12 [26].

3. Discussion

The fact that obesity is associated with increased risk for NTD and that most cases of NTD stem from lack of folate during critical days in the first postconceptional month, naturally leads to investigate folate status among obese women.

Indeed, there is strong evidence that overall, obese women consume less folic acid than women with normal BMI. However, there is also evidence that even with recommended levels of folate intake, the risk of NTD among offspring of obese women is higher than expected. This has led to a closer look how the obese body handles folic acid. Very few formal pharmacokinetic studies have been conducted, despite the importance of this question, showing that although the distribution volume of folate is larger among the obese, this cannot settle the scientific question. People commonly assume that if the volume of distribution is larger, then levels will be lower. But this is erroneous: distribution volume may be initially larger, but after this volume is filled, it is only the clearance rate of an agent and its given dose that determines its steady state levels, and not the distribution volume. As shown by Stern and colleagues, the overall clearance rate of folate is not different between obese and normal BMI women [87.9 (59.6–265.3) mL/min in normal vs. 90.7 (69.9–252.9) mL/min] [32]. But the story is more complex: while the obese women in Stern's study weighted on average 66% more, the clearance rate per Kg body weight is almost double in healthy vs. obese (1.52 mL/kg/min vs. 0.88 mL/kg/min). And this brings us to discuss the nature of body weight: total body weight (TBW) vs. lean body weight (LBW). Stern *et al.* have shown clearly that lean body weight predicts steady state serum concentrations of folate more accurately than total body weight. This implies that folate distributes much less into fat tissue than into lean body weight. Hence if folate dose is standardized per TBW, the woman may be proportionally overdosed. It is surprising that only little body of research has been published on folic acid dose optimization for obese women planning pregnancy. This question is highly relevant to millions of women worldwide, and measurements of folic acid are abundantly available. With numerous health organizations maintaining electronic health records, it should not be difficult to fully characterize the complex relationship between folic acid dose, folate serum and RBC levels, BMI and NTD.

Attempts to control for confounders have shown, for example, that in studies investigating obesity and the risk of NTD, even with adjustment for diabetes mellitus, the risk of obesity remained significant [21, 22]. In a similar manner, appropriate folate levels among febrile women mitigated the risk caused by fever itself, but did not eliminate it. Clearly, future work will have to better address confounders clustered among obese women. The new evidence suggesting lower folate transpoter expression and activity in obese mother, is an excellent example of biological confounders that much more fully addressed.

While the association between obesity and NTD is proven, as is the association between folic acid and NTD, the interaction between these two associations is much more complex.

It does appear that serum folate is consistently lower among obese women, but this does not appear true for RBC folate. Moreover, lower folate in obese women can be the result of lower intake, or higher pharmacokinetic needs. Stern *et al.* has provided evidence that lower folate levels cannot be simply explained by higher distribution volume, as fat tissue may be inert and the distribution of folate may not be predicted by higher BMI.

Future research will be needed to incorporate more fully, in addition to evidence of NTD, obesity and folic acid intake, also direct measurements of serum and RBC folate, as well as other confounders, in order to create a model that will shed more light on these questions.

Author contributions

GK conceptualised and wrote the manuscript. YCK contributed to the writing of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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