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Maternal choline status during pregnancy, but not that of betaine, is related to antenatal mental well-being: the Growing Up in Singapore Towards healthy Outcomes cohort

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Abstract

Background—Choline and betaine status have previously been associated with symptoms of depression. However, the relation of maternal plasma choline and betaine concentrations in pregnancy to peripartum maternal mood is unknown.

Methods—Maternal plasma choline and betaine concentrations (μmol/L) were measured at 26–28 weeks gestation in the GUSTO mother-offspring cohort. Participants completed the State-

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Authors' contributions: All authors were involved in all parts of the study and approved the final manuscript. LL and MFFC were responsible for study design, writing the manuscript and had primary responsibility for final content. LL performed statistical analyses. SMS, FKPY, KMG, YSC, and MJM designed and led the GUSTO study and provided intellectual contribution to the study and manuscript. PLQ and HC provided intellectual contribution to the study and manuscript.

Trait Anxiety Inventory (STAI) and Edinburgh Postnatal Depression Scale (EDPS) at 26-28 weeks gestation (n=949) and at 3 months postnatal (n=689): higher scores are indicative of more symptoms of anxiety and depression. Multivariate linear regression models were used to estimate the association of choline and betaine with ante- and postnatal mental well-being adjusting for covariates.

Results—Mean (SD) antenatal plasma choline and betaine concentrations were 9.2 $\mu\text{mol/L}$ (1.6) and 13.1 $\mu\text{mol/L}$ (2.7), respectively. Plasma choline concentrations were positively associated with antenatal depressive [$\beta=0.24$ EPDS score (95% CI 0.05, 0.43) per $\mu\text{mol/L}$] and anxiety symptoms [$\beta=0.46$ STAI-state score (95% CI 0.03, 0.88) per $\mu\text{mol/L}$] adjusting for covariates. Plasma betaine concentrations were not associated with antenatal depression or anxiety symptoms. No associations were observed between pregnancy choline or betaine and postnatal mental well-being.

Conclusion—This study suggests that higher maternal plasma choline status during pregnancy is associated with more symptoms of antenatal depression and anxiety, while plasma betaine concentrations showed no associations. No associations were observed for postnatal mental well-being. Prospective studies are required to replicate these findings and further examine the direction of causality and possible biological mechanisms.

Keywords

Choline; nutritional status; pregnancy depression; anxiety; mental well-being; peripartum period

Introduction

Approximately 13-19% of all mothers suffer from depression or anxiety during pregnancy or after (Gavin et al., 2005; O'Hara & Swain, 1996). This can lead to disturbing consequences for both the mother and her offspring. Perinatal depression has been associated with a higher risk of preeclampsia, loss of productivity, increased health care use, unfavorable parenting practices, impaired mother-infant bonding, and increased risks for depressive symptoms and central adiposity in the offspring (Andersson, Sundstrom-Poromaa, Wulff, Astrom, & Bixo, 2004; Gentile, 2017).

Neurotransmitter levels in the brain can be influenced by dietary nutrients; it has been shown that dietary choline intake can alter the neurotransmitter acetylcholine levels (Blusztajn & Wurtman, 1983). There is clinical evidence for a relation between levels of both choline and betaine and clinical status in depression. Choline is found in high concentrations in animal products such as meat, liver, and eggs (Zeisel, Mar, Howe, & Holden, 2003), and can be synthesized endogenously. Choline is also the precursor for betaine and phosphatidylcholine that are involved in methylation processes and membrane synthesis, respectively.

Choline is less investigated in relation to mental well-being as compared with nutrients associated with the synthesis of monoamines such as folate and tryptophan (Miller, 2008; Papakostas, Cassiello, & Iovieno, 2012; Réus et al., 2015). Results from studies examining choline and mental well-being have been contradictory and limited by sample size (Cohen, Lipinski, & Altesman, 1982; Davis, Hollister, & Berger, 1979; Stoll et al., 1996; Tamminga, Smith, Chang, Haraszti, & Davis, 1976; Vida, Gauthier, & Gauthier, 1989). Most studies

have focused on clinical patient groups (Cohen et al., 1982; Davis et al., 1979; Olvera et al., 2010; Stoll et al., 1996; Tamminga et al., 1976; Vida et al., 1989), such that the association between choline status and mood across the population is unknown. To date, choline supplementation reduced depressive mood symptoms in 6 patients with bipolar disorder (Stoll et al., 1996) and improved symptoms in 11 patients with mania (Cohen et al., 1982). A case-control study in adolescents with major depression reported lower brain phosphatidylcholine and glycerol-3-phosphocholine concentrations compared to healthy controls (Olvera et al., 2010), while a cross-sectional study reported inverse associations between plasma choline concentrations and anxiety levels in generally healthy men and women (Bjelland, Tell, Vollset, Konstantinova, & Ueland, 2009). In contrast, choline or lecithin (a source of choline) supplementation trials reported an increase in depressive symptoms in two patients with tardive dyskinesia (Tamminga et al., 1976) and nine patients with schizophrenia (Davis et al., 1979). Studies on betaine, an important methyl-donor, in relation to mental well-being are even scarcer. Two studies reported that betaine supplementation in combination with S-adenosylmethionine (SAME) was a more effective treatment than SAME alone in patients with mild-to-moderate depression (F. Di Pierro, Orsi, & Settembre, 2015; Francesco Di Pierro & Settembre, 2015).

No studies to date have been conducted in pregnant women, yet this may be important as pregnant women have a higher dietary choline requirement to meet the needs of the growing fetus (Institute of Medicine (IOM), 1998). Most pregnant women in an US sample were unable to meet these extra needs (Jensen, Batres-Marquez, Carriquiry, & Schallinske, 2007), potentially increasing their risk of deficiency-related disorders such as non-alcoholic fatty liver disease or birth defects (Zeisel, 2013) and initiating a discussion for the need of choline supplementation during pregnancy (Jiang, West, & Caudill, 2014; Zeisel, 2013).

The relation between nutrient levels and maternal mental well-being is an important research topic, as nutrient levels represent a highly realistic target for both prevention and intervention. The relationship between choline and betaine status during pregnancy and mental well-being has been largely unexplored. In this study, we, therefore, aimed to examine maternal choline and betaine concentrations during pregnancy in relation to ante- and postnatal depressive symptoms and anxiety in a large cohort of pregnant women in Singapore.

Methods

Study design and population

The Growing Up in Singapore Toward healthy Outcomes (GUSTO) study is a large multi-ethnic Asian mother-offspring cohort study aimed at evaluating the role of early-life exposures on later-life metabolic disease risks ([clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01174875); NCT01174875). Details on study aims and design have been described elsewhere (Soh et al., 2014). Briefly, 1247 pregnant women were recruited from two major maternity units in Singapore between June 2009 and September 2010. Participants were either Chinese, Malay, or Indian Singaporean citizens or permanent residents of which the parents and spouse's parents had to be from a homogenous ethnic background. Participants were eligible when aged between 19 and 50 years old, having the intention to deliver in the one of the study maternity units, be living in

Singapore for next 5 years and willing to donate cord, placenta, and cord blood at delivery. The major exclusion criterion was having preexisting serious health conditions including self-reported type I diabetes, psychoses or receiving chemotherapy or psychotropic drugs.. The study was approved by the medical ethical review boards of National University Hospital and KK Women's and Children's Hospital. Prior to recruitment, all participants gave written informed consent.

Maternal plasma nutrient concentrations

Blood samples at 26-28 weeks gestation were processed within 4 hours and stored at -80 °C prior to analysis. Plasma concentrations of free choline and betaine were analyzed by HPLC (1100 series, Agilent technologies) and tandem mass spectrometry (API 3000, Ab Sciex) (Midttun, Kvalheim, & Ueland, 2013). The within- and between-day imprecision coefficients of variation for these metabolites were between 4-10% (Midttun et al., 2013). Plasma folate concentrations were assessed by competitive electrochemiluminescence immunoassay (ADVIA Centaur Immunoassay System, Siemens): between-assay coefficients of variation were between 6-11%.

Maternal mental state

At 26-28 weeks gestation and at 3 months post-delivery, all participants self-administered the Edinburgh Postnatal Depression Scale (EPDS) and the State-trait Anxiety Inventory (STAI). The EPDS is a 10-item screening tool scored between 0-3 that rates the intensity of depressive symptoms present in the past seven days (Cox, Holden, & Sagovsky, 1987). EPDS is considered valid for use in multiple cultures during and after pregnancy (Gibson, McKenzie-McHarg, Shakespeare, Price, & Gray, 2009; Kozinszky & Dudas, 2015). Pregnant women with antenatal EPDS score ≥ 15 was considered as having probable depression during pregnancy, whereas postnatal EPDS score ≥ 13 indicated a probable depression postpartum (Chen et al., 2011; Gibson et al., 2009). The term probable depression was used, as the EPDS examines mood in terms of symptom severity rather than clinical diagnoses.

The STAI consists of two subscales (State and Trait anxiety) with each 20 items scored 0-4 to assess anxiety levels (Spielberger, Gorsuch, Lushene, Vagg, & Jacobs, 1983). The STAI has shown construct validity (Meades & Ayers, 2011) and was considered reliable within the GUSTO cohort (Qiu et al., 2013). STAI-state reflects a transitory emotional state and may, therefore, fluctuate over time, whereas STAI-trait represents a relatively stable individual tendency of anxiety. In the present study only the STAI-state measures were used. The top 75th percentiles of the study sample was used to define high levels of anxiety (STAI-state antenatal score ≥ 41 and postnatal score ≥ 40), as suggested by others (Nasreen, Kabir, Forsell, & Edhborg, 2010; Teixeira, Fisk, & Glover, 1999).

Covariates

Ethnicity and maternal age were collected during the recruitment phase at 12-14 weeks of gestation. Detailed information on demographics and maternal lifestyle including smoking habits, income, and educational level were collected during the clinic visit at 26-28 weeks of gestation. Educational level was categorized in low, intermediate, and high, and income was

categorized as <\$1999, 2000-5999, >\$6000. Regular pre-pregnancy smokers (y/n) were defined as smoking once a day for a year or longer.

Statistical analyses

Out of 1247 recruited pregnant women, 949 had their plasma concentrations of choline and betaine measured and had complete data for antenatal STAI and EPDS (Figure 1). For the postnatal analyses, 689 participants had complete data for nutrient markers, STAI and EPDS.

Maternal characteristics were presented according to antenatal probable depression and anxiety. P values for difference were computed using an independent t-test for continuous variables and F-test statistic for categorical variables. Linearity of the associations were first examined by use of restricted cubic splines and they indicated linear associations (all $P_{\text{non-linearity}} > 0.10$). We then modeled EPDS and STAI-state scores continuously using linear regression models. To study a dose-response relationship, we used Poisson regression models with robust variance to calculate the prevalence risk ratios (PR) of having probable depression or anxiety according to tertiles (Choline: T1 5.4-8.3 $\mu\text{mol/L}$, T2 8.3-9.7 $\mu\text{mol/L}$, T3 9.7-14.8 $\mu\text{mol/L}$; Betaine: T1 5.3-11.9 $\mu\text{mol/L}$, T2 11.9-14.0 $\mu\text{mol/L}$, T3 14.0-25.0 $\mu\text{mol/L}$). PRs were used because odds ratios could overestimate the strength of the association in non-case-control studies when the disease is not rare (Coutinho, Scazufca, & Menezes, 2008; Thompson, Myers, & Kriebel, 1998). Linear trends were modeled using the median of tertiles as a continuous variable in the model. We presented the following three models: Model 1: unadjusted; Model 2: adjusting for regular pre-pregnancy smoking, maternal age, ethnicity, educational level, and income; Model 3: adjusting for confounders in model 2, plus plasma folate and choline or betaine concentrations. When analyzing postnatal mental well-being, we additionally included antenatal mental state scores as a covariate. In sensitivity analyses, we further studied the potential confounding effects of plasma total omega-3, total omega-6 fatty acid concentrations ($\mu\text{g/mL}$) (Chong et al., 2015) and alcohol use (y/n) (Moss, Goldstein, Chen, & Yi, 2015). In addition, we included an interaction term of nutrient status with ethnicity and folate status in the relationship with antenatal mental well-being. Lastly, correlations between plasma choline concentrations and food groups as assessed by a 24-hour recall at 26-28 weeks gestation were examined to explore the dietary sources of choline in this study sample.

Missing values for income (n=63), educational level (n=16), pre-pregnancy smoking (n=1), and folate concentration (n=1) were imputed for 20 times and results were pooled. All analyses were performed in STATA version 14.1 (StataCorp LP, USA) and statistical significance was set at $P < 0.05$.

Results

Mean choline and betaine concentrations in the antenatal study sample were 9.2 $\mu\text{mol/L}$ (SD 1.6) and 13.1 $\mu\text{mol/L}$ (SD 2.7), respectively. The prevalence of probable depression and mean (SD) EPDS scores in our study sample was 7% and 7.4 (4.5) respectively during the antenatal period and 10% and 6.3 (4.7) respectively during the postnatal period. For probable anxiety, the prevalence and mean (SD) STAI-state scores was 27% and 33.9 (10.0)

respectively during the antenatal period and 25% and 33.7 (10.2) respectively during the postnatal period. Women who suffered from antenatal probable depression had higher plasma betaine concentrations, lower plasma folate concentrations, were younger, more likely to smoke regularly before pregnancy, had a lower education level and a lower income compared to non-depressive women (Table 1). Mean choline concentrations were similar among the women with (9.4 ± 1.7) and without (9.1 ± 1.6 ; $P=0.195$) probable depression. Women, who had probable antenatal anxiety had higher plasma choline concentrations, were younger, more likely to smoke regularly before pregnancy and had a lower educational level, income and plasma total omega-3 concentrations. When examining the postnatal study sample, we observed similar trends in the sample characteristics (Supplemental table 1).

Choline and mental well-being

Linear regression models showed statistically significant positive associations between antenatal plasma choline concentrations and antenatal depression scores in crude and multivariate adjusted models [model 3: $\beta=0.24$ EPDS score (95% CI 0.05-0.043) per $\mu\text{mol/L}$; Table 2]. Similarly, choline showed a positive trend with antenatal anxiety in unadjusted models and this reached significance after including all covariates [model 3: $\beta=0.46$ STAI-state score (95% CI 0.03, 0.88) per $\mu\text{mol/L}$].

In the dose-response analyses, the mean scores for EPDS and STAI-state differed significantly across tertiles of choline during pregnancy ($P_{\text{EPDS}}=0.006$; $P_{\text{STAI-state}}=0.021$; Table 3), but not postpartum ($P_{\text{EPDS}}=0.206$; $P_{\text{STAI-state}}=0.652$; Table 4). The pregnant women in the highest tertile of choline, as compared to those in the lowest tertile, had a trending higher risk of antenatal probable depression [$\text{PR}=1.76$ (95% CI 0.97, 3.18) $P_{\text{trend}}=0.065$]. This association attenuated after adjusting for plasma folate and betaine, although effect sizes were similar. Women in the highest tertile of choline, as compared to those in the lowest tertile, had a significant 37% higher risk of antenatal probable anxiety [$\text{PR}=1.37$ (95% CI 1.04, 1.82) $P_{\text{trend}}=0.029$], which attenuated to a trending association after including all covariates [Model 3: $\text{PR}=1.36$ (95% CI 1.00, 1.85); Table 3]. No associations were observed between tertiles of choline and postnatal probable depression [Model 3: $\text{PR}_{\text{T3 vs. T1}}=1.16$ (95% CI 0.65, 2.07) $P_{\text{trend}}=0.594$] or postnatal probable anxiety [$\text{PR}_{\text{T3 vs. T1}}=1.16$ (95% CI 0.83, 1.61) $P_{\text{trend}}=0.472$].

Betaine and mental well-being

Using linear regression models, we observed a trending positive association between plasma betaine and depression scores [$\beta=0.10$ EPDS score (95% CI 0.00-0.21) per $\mu\text{mol/L}$] (Table 2), which disappeared after including covariates. No associations were observed between plasma betaine concentrations and antenatal anxiety or postnatal mental well-being. The mean scores for EPDS and STAI-state were similar across tertiles of betaine during pregnancy ($P_{\text{EPDS}}>0.147$; $P_{\text{STAI-state}}=0.799$; Table 3) and postpartum ($P_{\text{EPDS}}>0.723$; $P_{\text{STAI-state}}=0.724$; Table 4). When comparing the highest betaine concentrations to the lowest, we observed no associations for antenatal (Table 3) or postnatal mental well-being [depression: $\text{PR}_{\text{T3 vs. T1}}=0.70$ (95% CI 0.40, 1.22) $P_{\text{trend}}=0.178$; anxiety: $\text{PR}_{\text{T3 vs. T1}}=0.90$ (95% CI 0.66, 1.23) $P_{\text{trend}}=0.597$], while adjusting for all covariates.

Sensitivity analyses

Sensitivity analyses showed no effect modification of ethnicity or plasma folate status in the relationship between plasma choline or betaine status on ante- and postnatal mental well-being. Additional adjustment for alcoholic beverage use, plasma total omega-3 or total omega-6 fatty acid concentrations did not change our results significantly (data not shown). We found positive correlation coefficients between plasma choline and dietary intake of eggs ($r=0.17$), bread ($r=0.13$), dairy ($r=-0.10$), fish ($r=0.07$), and poultry ($r=0.02$).

Discussion

To our knowledge, this is the first study to report on the relation between higher antenatal plasma choline concentrations and antenatal depression and anxiety (mental well-being) in a peripartum cohort. No associations were observed between plasma betaine and antenatal mental well-being, as well as between choline or betaine concentrations and postnatal mental well-being.

Our choline and betaine concentrations were comparable to previously reported concentrations, which varied between 7.0-9.4 $\mu\text{mol/L}$ for plasma choline and 10.3-11.0 $\mu\text{mol/L}$ for plasma betaine at 24-29 weeks of gestation (Velzing-Aarts et al., 2005; Yan et al., 2012). Due to the lack of established cut-off values defining plasma choline and betaine deficiency, it remains unclear whether our plasma concentrations represent adequate intakes.

The positive association between plasma choline concentration and mental well-being observed in this study supports the results observed in animal and human trials (Dagyte, Den Boer, & Trentani, 2011; Davis et al., 1979; Janowsky, Overstreet, & Nurnberger, 1994; Mineur et al., 2013; Philip, Carpenter, Tyrka, & Price, 2010; Tamminga et al., 1976). These trials either supplemented with choline or increased neural acetylcholine levels by inhibition or knock-out of the breakdown of neural acetylcholine (i.e. cholinesterase) or by blocking acetylcholine receptors. Unfortunately, no choline concentrations were reported in these studies and thus direct comparison with our concentrations was not possible.

In contrast to our findings, choline supplementation has been shown to improve depression and mania scores in patients with mental disorders (Brown & Gabrielson, 2012; Cohen et al., 1982; Stoll et al., 1996). These trials administered 2-6 g choline/day, which corresponds to 4 to 12 times the adequate intake of dietary choline of 450 mg/day. These doses are likely to be considerably higher compared to the dietary choline intake in our study. Strikingly, a cross-sectional analysis in middle-aged and elderly adults with similar plasma choline concentrations (range 4.1-24.7 $\mu\text{mol/L}$) reported an inverse association between plasma choline concentrations and anxiety as measured with the Hospital Anxiety and Depression scale (Bjelland et al., 2009). These associations were, however, not observed in women after stratification for sex.

Unlike other methyl-donor nutrients such as folate and SAMe that have been shown to be inversely associated with depressive symptoms (Miller, 2008; Papakostas et al., 2012), we did not observe a significant association between betaine and antenatal or postnatal mental well-being. Our finding was supported by the results from a cross-sectional study in

Norwegian adults that also observed no associations between plasma betaine concentrations and mental well-being (Bjelland et al., 2009).

Mechanism

Depression has been considered a complex and multi-factorial disease of unknown etiology. However, research in animals and humans has elucidated some insight into possible mechanisms through which choline might exert its effect on depressive symptoms and anxiety levels.

A plausible mechanism through which choline may play a role in depression and anxiety is via neural acetylcholine concentrations. Cholinesterase inhibitors, which inhibit breakdown of acetylcholine in extracellular space, and acetylcholine receptor antagonists, induced depressive symptoms, presumably by increasing neural acetylcholine (Dagyt et al., 2011; Janowsky et al., 1994). These results were confirmed in mice studies showing that local administration of physostigmine or knockdown of acetylcholinesterase increased anxiety-like and depression-like behaviors (Mineur et al., 2013). Additionally, choline supplementation upregulated $\alpha 7$ nicotinic acetylcholine receptor levels in the hippocampus of rats, whereas stress altered levels of these nicotinic acetylcholine receptor levels (Schulz et al., 2014).

Alterations in the hypothalamic-pituitary-adrenal (HPA) axis might also play a role. Acetylcholine receptors, which are located on the presynaptic terminals of corticotrophin releasing factor neurons, have shown to affect key components of the HPA axis and HPA axis function alterations such as increased cortisol and adrenocorticotrophic hormone release have been seen in depressed patients (Janowsky & Overstreet, 2000; Philip et al., 2010). In healthy and psychiatric patients, physostigmine, a cholinesterase inhibitor, has shown to induce HPA axis resulting in increases in ACTH, β -endorphin and cortisol immunoreactivities (Janowsky & Risch, 1984; Risch, Janowsky, & Gillin, 1983). Furthermore, mecamylamine, an acetylcholine receptor antagonist, can prevent corticotropin releasing factor release (Raber, Koob, & Bloom, 1995). Moreover, in rats on a choline deficient diet, the HPA response to auditory stress was impaired which was partially corrected by choline supplementation (Sithichoke, Malasanos, & Marotta, 1978). Lastly, higher maternal dietary choline intake (930 mg/day versus 480 mg/day) lowered offspring circulating cortisol by altering the methylation state of cortisol-regulating genes in the placenta and fetal compartments (Jiang et al., 2014).

Another plausible mechanism might be via mitochondrial dysfunction that occurs in certain more serious neuropsychiatric disorders (Morris & Berk, 2015; Müller et al., 2015), whereby more choline may be available for transformation into phospholipids or acetylcholine as choline is oxidized to betaine in the mitochondrion. Patients with mitochondrial dysfunction showed higher levels of hippocampal glycerol-3-phosphocholine, a type of phospholipid, than controls (Anglin, Rosebush, Noseworthy, Tarnopolsky, & Mazurek, 2012). Higher levels of glycerol-3-phosphocholine have also been associated with anxiety and bipolar disorders (Anglin et al., 2012; Senaratne, Milne, MacQueen, & Hall, 2009).

Strengths and limitations

Important strengths of the present study were the fairly large sample size and the availability of plasma folate, vitamin B12 and omega-3 fatty acids concentrations. These nutrients were included in our analyses, because they have previously been associated with depression and anxiety (Chong et al., 2015; Chong et al., 2014). Furthermore, we used plasma choline and betaine concentrations, thus eliminating the possibility of recall bias of dietary intake as compared to commonly-used dietary assessment methods (Kipnis et al., 2003). These plasma concentrations reflect both dietary intake and endogenous syntheses. This said, a limitation is that we are uncertain about the main dietary sources of choline. It is likely that our reported choline concentrations were influenced by dietary intake, as most prenatal supplements do not contain sources of choline (Zeisel, 2013). Two studies in Asians have reported eggs, pork, chicken, fish, soy foods, dairy, and vegetables to be key food sources contributing to dietary choline intake (Chu, Wahlqvist, Chang, Yeh, & Lee, 2012; Yu et al., 2014). We found positive correlation coefficients with similar food sources in our cohort. Next to dietary sources, plasma choline concentrations may be influenced by gut microbiota, as some evidence suggests the existence of acetylcholine-producing bacteria and the breakdown of choline into trimethylamine (Sandhu et al., 2017; Smallwood, Allayee, & Bennett, 2016). Unfortunately no data on microbiome in mothers was available to further examine these possible interactions with our plasma choline concentrations. Another important limitation was the partial cross-sectional design of our study that limits causal inferences. Consequently, we cannot exclude the possibility of a bi-directional relationship where depressive symptoms during pregnancy lead to an altered diet and subsequent nutrient status. In addition, the plasma markers were only available at 26-28 weeks of gestation and may not accurately reflect postnatal status given that choline levels have previously shown to increase during pregnancy and decrease after birth (Velzing-Aarts et al., 2005). Multiple assessments of these plasma markers over the course of pregnancy could provide useful insight into how the maternal choline and betaine levels across pregnancy may affect the outcome of our study differently. Moreover, as all observational studies, we cannot exclude the possibility of residual confounding by unmeasured or poorly measured covariates. For example, no information was available on possible changes in anti-depressive drug use. Nevertheless, we included many covariates known for their association with depression, and choline or betaine. Lastly, as we excluded persons who self-reported pre-existing mental disorders, results of our study might not be generalizable to populations with more severe symptoms of depression and anxiety. Further research in clinically depressed or anxious women during the perinatal period is warranted.

Implications and future research

We observed that higher antenatal plasma choline concentrations were associated with more depression and anxiety symptoms during pregnancy, whereas plasma betaine concentrations showed no associations. Our findings add information to the discussion for the potential need for choline supplementation during pregnancy (Jiang et al., 2014; Zeisel, 2013) and suggest caution for relative high choline concentrations in relation to mental health. Prospective studies are required to replicate these findings and study the causality of these associations. More research is, moreover, needed to investigate potential dietary food

sources that contribute to higher choline concentrations during pregnancy for future dietary interventions and potential prevention of mental illnesses. Lastly, future studies should also focus on the subsequent effect of maternal choline status on offspring mental well-being, as it recently has been shown in an animal study that perinatal maternal choline supplementation could ameliorate the effects of prenatal stress on anxiety-related behavior in offspring (Schulz et al., 2014).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Flowchart of study participants at 26-28 weeks of gestation and at 3 months postpartum

Table 1

Study sample characteristics^a according to probable antenatal depression and anxiety in 949 participants of the GUSTO cohort

	Normal (n=880)	Probable depression ^b (n=69)	P value ^c	Normal (n=707)	Probable anxiety ^b (n=242)	P value ^c
Choline (μmol/L)	9.1 ± 1.6	9.4 ± 1.7	0.195	9.1 ± 1.6	9.3 ± 1.7	0.035
Betaine (μmol/L)	13.1 ± 2.6	13.8 ± 3.3	0.044	13.1 ± 2.6	13.4 ± 2.8	0.129
plasma folate (nmol/L)	34.7 (20.5)	29.5 (24.1)	0.017	34.6 (20.1)	33.6 (20.4)	0.522
Maternal age (y)	30.8 ± 5.0	28.9 ± 6.5	0.004	30.9 ± 4.9	29.8 ± 5.8	0.002
Maternal pre-pregnancy BMI (kg/m ²)	22.6 ± 4.3	22.9 ± 5.0	0.576	22.5 ± 4.2	22.8 ± 4.6	0.354
Pre-pregnancy regular smoking	11.7	31.9	<0.001	10.6	20.7	<0.001
Physical active	28.0	23.5	0.426	29.1	23.6	0.095
Marital status	96.4	95.2	0.594	96.8	95.4	0.305
Educational level			<0.001			<0.001
Pri and secondary	29.2	51.4		25.7	45.5	
Post-secondary	35.2	30.9		35.2	34.2	
University	35.6	17.6		39.1	20.3	
Income			<0.001			<0.001
S\$1999	14.6	29.9		12.4	25.3	
S\$2000-5999	54.8	63.5		53.7	60.5	
S\$6000	30.6	6.6		33.9	14.2	
Ethnicity			0.001			0.061
Chinese	57.2	33.3		57.3	50.0	
Malay	25.2	39.1		24.3	31.8	
Indian	17.6	27.5		18.4	18.2	
Alcohol use	2.2	0.0	0.215	2.3	1.3	0.332
Total omega-3 (μg/mL)	139.5 (93.1)	126.8 (86.8)	0.714	143.5 (94.9)	122.3 (83.5)	0.005
Total omega-6 (μg/mL)	789.5 (384.9)	760.2 (347.8)	0.714	793.7 (385.6)	763.7 (361.6)	0.382

BMI, body mass index

^aCharacteristics are presented in mean ± SD, median (IQR) or as percentages

^bAntenatal probable depression EPDS 15; Antenatal probable anxiety STAI-state 41

^cTest for difference is calculated with F test.

Table 2

Multivariate linear regressions of antenatal plasma choline and betaine status with depression and anxiety scores in 949 participants of the GUSTO cohort.

	Depression scores (EPDS)						Anxiety scores (STAI-state)					
	Antenatal			Postnatal ^a			Antenatal			Postnatal ^a		
	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value	β	95% CI	P value
Choline												
Model 1	0.23	0.05, 0.40	0.010	0.10	-0.10, 0.30	0.335	0.35	-0.04, 0.74	0.075	-0.01	-0.45, 0.42	0.956
Model 2	0.24	0.07, 0.41	0.005	0.09	-0.11, 0.29	0.364	0.37	-0.09, 0.75	0.055	-0.05	-0.49, 0.38	0.812
Model 3	0.24	0.05, 0.43	0.021	0.08	-0.13, 0.28	0.459	0.46	0.03, 0.88	0.036	-0.20	-0.66, 0.26	0.393
Betaine												
Model 1	0.10	0.00, 0.21	0.059	-0.04	-0.17, 0.08	0.484	0.07	-0.17, 0.31	0.570	0.02	-0.25, 0.29	0.882
Model 2	0.07	-0.04, 0.17	0.198	-0.05	-0.18, 0.07	0.401	0.01	-0.23, 0.24	0.939	-0.01	-0.28, 0.26	0.957
Model 3	0.03	-0.09, 0.14	0.667	-0.09	-0.22, 0.03	0.153	-0.09	-0.36, 0.17	0.495	0.09	-0.20, 0.38	0.556

EPDS: Edinburgh Postnatal Depression Scale, STAI: State-Trait Anxiety Inventory

Model 1: Crude

Model 2: Adjusted for pre-pregnancy regular smoking (y/n), maternal age (y), ethnicity (Chinese, Malay, Indian), educational level (low, intermediate, high), income (S\$1999, S\$2000-5999, S\$6000).

Model 3: model 2 and additionally adjusted for plasma folate (nmol/L) and choline or betaine concentrations ($\mu\text{mol/L}$)

^a Additionally adjusted for antenatal scores, n=689

Table 3

Multivariate prevalence ratios of antenatal plasma choline and betaine concentrations with antenatal probable depression and anxiety in 949 participants of the GUSTO cohort.

			Antenatal probable depression ^a								
Cases	Mean EPDS (SD)	Model 1			Model 2			Model 3			
		PR	95% CI	P value	PR	95% CI	P value	PR	95% CI	P value	
Choline ^b											
Tertile 1	16	6.8 (4.3)	1.00	reference	-	1.00	reference	-	1.00	reference	-
Tertile 2	25	7.4 (4.6)	1.57	0.85, 2.88	0.147	1.73	0.96, 3.10	0.066	1.68	0.94, 3.00	0.081
Tertile 3	28	7.9 (4.6)	1.76	0.97, 3.18	0.063	1.76	0.99, 3.15	0.056	1.70	0.91, 3.16	0.094
<i>P for trend</i>					0.065	0.061			0.121		
Betaine ^c											
Tertile 1	20	7.1 (4.4)	1.00	reference	-	1.00	reference	-	1.00	reference	-
Tertile 2	17	7.2 (4.3)	0.95	0.51, 1.78	0.871	0.94	0.50, 1.74	0.813	0.95	0.50, 1.82	0.888
Tertile 3	32	7.8 (4.8)	1.69	0.99, 2.89	0.055	1.48	0.86, 2.55	0.153	1.45	0.81, 2.59	0.211
<i>P for trend</i>					0.052	0.137			0.168		
Mean STAI-state (SD)			Antenatal probable anxiety ^d								
Choline ^b											
Tertile 1	65	32.7 (9.9)	1.00	reference	-	1.00	reference	-	1.00	reference	-
Tertile 2	88	34.8 (9.9)	1.36	1.03, 1.80	0.032	1.44	1.10, 1.89	0.009	1.45	1.10, 1.92	0.009
Tertile 3	89	34.3 (10.4)	1.37	1.04, 1.82	0.026	1.37	1.04, 1.81	0.023	1.36	1.00, 1.85	0.053
<i>P for trend</i>					0.029	0.029			0.070		
Betaine ^c											
Tertile 1	78	33.7 (9.8)	1.00	reference	-	1.00	reference	-	1.00	reference	-
Tertile 2	73	33.9 (9.5)	1.05	0.79, 1.38	0.754	1.05	0.80, 1.38	0.698	0.96	0.73, 1.28	0.802
Tertile 3	91	34.2 (10.7)	1.23	0.95, 1.60	0.115	1.15	0.89, 1.49	0.269	1.02	0.78, 1.35	0.883
<i>P for trend</i>					0.113	0.266			0.827		

Model 1: Unadjusted

Model 2: Adjusted for pre-pregnancy regular smoking (y/n), maternal age (y), ethnicity (Chinese, Malay, Indian), educational level (low, intermediate, high), income (<\$1999, 2000-5999, >\$6000) and antenatal scores

Model 3: Model 2 and additionally adjusted for plasma folate (nmol/L) and plasma betaine or choline concentrations (μmol/L)

^a Antenatal probable depression was defined as Edinburgh Postnatal Depression Scale 15

^b Choline: T1 5.4-8.3 μmol/L, T2 >8.3-9.7 μmol/L, T3 >9.7-14.8 μmol/L

^c Betaine: T1 5.3-11.9 μmol/L, T2 >11.9-14.0 μmol/L, T3 >14.0-25.0 μmol/L

^d Antenatal anxiety was defined as State-Trait Anxiety Index 41

Table 4

Multivariate prevalence ratios of antenatal plasma choline and betaine status with postnatal probably depression and anxiety in 689 participants of the GUSTO cohort.

			Postnatal probable depression ^a								
			Model 1			Model 2			Model 3		
Cases	Mean EPDS (SD)		PR	95% CI	P value	PR	95% CI	P value	PR	95% CI	P value
Choline ^b											
T1	22	5.8 (4.6)	1.00	reference	-	1.00	reference	-	1.00	reference	-
T2	23	6.5 (4.9)	1.11	0.63, 1.93	0.721	1.03	0.60, 1.77	0.906	1.07	0.62, 1.84	0.820
T3	25	6.5 (4.6)	1.17	0.68, 2.01	0.580	1.05	0.62, 1.77	0.869	1.16	0.65, 2.07	0.616
<i>P for trend</i>					0.582	0.872			0.594		
Betaine ^c											
T1	26	6.4 (5.0)	1.00	reference	-	1.00	reference	-	1.00	reference	-
T2	22	6.4 (9.9)	0.97	0.56, 1.65	0.901	0.90	0.54, 1.52	0.705	0.93	0.54, 1.60	0.784
T3	22	6.1 (4.6)	0.86	0.50, 1.48	0.585	0.68	0.41, 1.16	0.156	0.70	0.40, 1.22	0.208
<i>P for trend</i>					0.582	0.148			0.178		
Mean STAI-state (SD)			Postnatal probable anxiety ^d								
Choline ^b											
T1	54	33.2 (10.0)	1.00	reference	-	1.00	reference	-	1.00	reference	-
T2	66	34.0 (10.3)	1.29	0.95, 1.76	0.104	1.21	0.90, 1.62	0.199	1.24	0.92, 1.68	0.154
T3	64	34.0 (10.4)	1.22	0.89, 1.66	0.221	1.09	0.81, 1.48	0.555	1.16	0.83, 1.61	0.379
<i>P for trend</i>					0.242	0.617			0.472		
Betaine ^c											
T1	66	33.7 (10.6)	1.00	reference	-	1.00	reference	-	1.00	reference	-
T2	51	33.3 (9.9)	0.88	0.64, 1.21	0.438	0.86	0.64, 1.17	0.350	0.84	0.60, 1.17	0.299
T3	57	34.1 (10.1)	1.03	0.77, 1.38	0.829	0.94	0.71, 1.24	0.644	0.90	0.66, 1.23	0.512
<i>P for trend</i>					0.815	0.669			0.597		

Model 1: unadjusted

Model 2: adjusted for pre-pregnancy regular smoking (y/n), maternal age (y), ethnicity (Chinese, Malay, Indian), educational level (low, intermediate, high), income (<\$1999, 2000-5999, >\$6000) and antenatal scores

Model 3: model 2 and additionally adjusted for plasma folate (nmol/L) and plasma betaine (μmol/L) or choline (μmol/L) concentrations

^aPostnatal probable depression was defined as Edinburgh Postnatal Depression Scale 13

^bCholine: T1 5.4-8.3 μmol/L, T2 >8.3-9.7 μmol/L, T3 >9.7-14.8 μmol/L

^cBetaine: T1 5.3-11.9 μmol/L, T2 >11.9-14.0 μmol/L, T3 >14.0-25.0 μmol/L

^dPostnatal probable anxiety was defined as State-Trait Anxiety Index 40