**Relation of plasma tryptophan concentrations during pregnancy to maternal sleep and mental well-being: The GUSTO cohort**

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**Introduction**

Pregnant women commonly report sleep difficulties, which is in part due to the many physical and psychologic changes during pregnancy. A prevalence ranging between 50-80% has been reported for poor sleep quality or insomnia in pregnant women (Ko et al., 2012; Sivertsen et al., 2015). This prevalence is concerning because poor sleep quality has been associated with, gestational diabetes (Cai et al., 2017), offspring preterm birth, delays in childhood development (Facco et al., 2012; Zhou et al., 2016) and postnatal psychological distress (Tham et al., 2016). In adults, poor sleep quality has been associated with poor daytime functioning, obesity, type 2 diabetes, cardiovascular disease and all-cause mortality (Dashti et al., 2015).

Symptoms of depression and anxiety are also common in the peripartum period, with a prevalence of 13-19% for a diagnosis of depression or anxiety disorders (Ko et al., 2012; Sivertsen et al., 2015), and even higher prevalence’s for symptoms of depression and anxiety (Norhayati et al.; Tham et al., 2016), and these have been associated with poorer maternal and offspring health (Andersson et al., 2004; Gentile, 2017; Qiu et al., 2015).

Poor sleep is a hallmark feature of mood disorders, including depression and anxiety (American Psychiatric Association, 2013; Mellor et al., 2014; Roth, 2007), probably due to common causes underlying these conditions (Staner, 2010). Nevertheless, there is evidence that insomnia can be distinguished from depressive disorders (Staner, 2010).

Tryptophan is an essential amino acid and a precursor of serotonin (5-hydroxytryptamine) and consequently melatonin; substances that are considered important in the modulation of several essential behaviors and psychological functions including sleep, mood, cognition, and circadian rhythms (Silber and Schmitt, 2010). The relationship between tryptophan and sleep quality has, to date, only been investigated in intervention trials and not in observational studies. A review including 21 trials in humans with and without sleep disorders concluded that increasing brain tryptophan by dietary treatments appeared to improve subjective measures of sleepiness and reduce sleep latency (i.e. time span between bedtime and sleep onset) (Silber and Schmitt, 2010). In addition, a 7-day trial with tryptophan-enriched cereals at breakfast and dinner showed beneficial effects on sleep/wake cycle in 35 elderly with sleep problems (Bravo et al., 2013). In 13 adults with recovered depression, tryptophan depletion by a tryptophan-free drink was associated with shorter rapid eye movement (REM) latency (i.e. time span between bedtime and start of REM sleep) as compared to the control drink (Haynes et al., 2004).

The evidence relating tryptophan to mental well-being is presently mixed. A meta-analysis and Cochrane review concluded that supplementation with tryptophan or serotonin significantly improved depressive symptoms as compared to the placebo treatment in patients with depression (Shaw et al., 2002a, b). This was further confirmed in a mega-analysis of five trials concluding that acute tryptophan depletion evokes depressive symptoms in 50% of the remitted depressed patients (Booij et al., 2002). However, a separate review of 13 trials in healthy and depressed adults, indicated mixed results and no clear conclusion (Silber and Schmitt, 2010).

To date, the relations between tryptophan and sleep quality and mental well-being have not been examined in healthy pregnant women, even though sleep problems and mental health problems were found to be more common in this group. Use of dietary tryptophan could provide a realistic target for both prevention and treatment of mental health and sleep problems in this group. However, observational studies are first warranted to study if associations exist during the perinatal period. Here, we will use data from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) cohort to examine this.

**Methods**

*Study design and participants*

The GUSTO cohort aims to evaluate the role of early-life exposures on later-life metabolic disease and neurodevelopmental risks in mother-offspring dyads. Detailed information on the study design and measurements has been reported (Soh et al., 2014). Eligible participants were either Chinese, Malay, or Indian Singaporean citizens or permanent residents, aged between a range of 18 and 50 years old, with the intention to deliver in one of the study maternity units, residing in Singapore for the next 5 years and willing to donate cord, placenta, and cord blood at delivery. Exclusion criteria were having preexisting self-reported type I diabetes, psychoses or receiving chemotherapy or psychotropic drugs. The study was approved by the National Healthcare Group Domain Specific Review Board (reference number D/09/021) and the Sing Health Centralized Institutional Review Board (reference number 2009/280/D). All participants gave written informed consent.

*Blood concentrations measurements*

At 26-28weeks gestation, participants underwent a venipuncture in fasting state during a clinic visit. Information on timing of the venipuncture was not recorded. The blood samples were centrifuged and stored at -80 °C before they were sent for analyses at the BEVITAL laboratory, Bergen, Norway. Plasma tryptophan, kynurenine, and pyridoxal 5’-phosphate (PLP) were analyzed using tandem mass spectrometry (API 4000, AB Sciex). The ratio between kynurenine and tryptophan may represent the flux through the tryptophan oxidation pathway over serotonin synthesis (Maes et al., 2002) and PLP is a rate-limiting cofactor in the synthesis of serotonin (Shabbir et al., 2013) and subsequently has been associated with mental well-being (Skarupski et al., 2010).

Within-day and between-day coefficients of variation for these markers ranged between 3-5% for tryptophan and kynurenine and 6-11% for PLP (Midttun et al., 2009). More detailed information on the analyses can be found elsewhere (Midttun et al., 2009).

*Sleep and mental well-being measurements*

Sleep and mental well-being were measured using self-administered questionnaires during the clinic visit at 26-28 weeks gestation and 3 months post. The Pittsburgh Sleep Quality Index was administered to estimate sleep quality (Buysse et al., 1989). It contains 19 items that generate 7 subcomponents scores (i.e. subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, sleep medication and daytime functioning) on a 0-3 scale and a summed global score ranging from 0 to 21; higher scores represent poorer subjective sleep quality. Subjective poor sleep quality was defined as having a global score > 5, which previously showed good sensitivity and specificity in patients with insomnia (Backhaus et al., 2002; Buysse et al., 1989).

Mental well-being was measured using 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 et al., 1987) and has been considered valid for use in multiple cultures during and after pregnancy (Gibson et al., 2009; Kozinszky and Dudas, 2015). Probable antenatal depression was defined as having an EPDS score ≥ 15, whereas postnatal EPDS scores ≥ 13 indicated probable depression postpartum (Chen et al., 2011; Gibson et al., 2009). The STAI consists of two 20-item subscales (State and Trait anxiety) scored 0-4 to assess anxiety levels and has been shown to have construct validity (Meades and Ayers, 2011) and reliability within the GUSTO study (Qiu et al., 2013). The current analyses only use the STAI-state measures because it reflects a transitory emotional state and not the anxious personality traits. The top 75th percentiles of the study sample were used to define probable anxiety in the antenatal (score ≥ 41) and postpartum (score ≥ 40) periods, as previously suggested by others (Nasreen et al., 2010; Teixeira et al., 1999).

*Covariates*

Information on demographics, characteristics, and lifestyle including age, ethnicity, smoking habits, physical activity and household income were collected using questionnaires at recruitment and during the clinic visit at 26-28 weeks gestation (Soh et al., 2014). Monthly household income was categorized as ≤S$1999, 2000-5999, ≥S$6000 and regular pre-pregnancy smokers (y/n) were defined as smoking once a day for a year or longer. Physical exercise during pregnancy was obtained for strenuous, moderate, and gentle activities and women were categorized into those who did or did not undertake moderate-to-strenuous physical activity (y/n). Predominantly nighttime feeding was defined as consuming more than 50% of total energy intake between 19.00h to 06.59h and was determined from a 24-hour recall conducted at 26-28 weeks of gestation (Loy et al., 2016).

*Statistical analyses*

Of the 1247 recruited pregnant women, 980 participants had information on plasma tryptophan concentrations (**Figure 1**). Participants who did not have complete data on sleep (n=395) or antenatal mental well-being (n=3) were excluded. Additionally, we excluded participants who reported a longer sleep duration than their time spent in bed (difference >|2| hours; n=10), which resulted in a sample of 572 participants. For the postnatal analyses, we excluded 326 participants who did not have complete data for postnatal PSQI, EPDS, or STAI resulting in a sample of 246 for these analyses.

Participants’ characteristics were reported according to their sleep quality (poor vs. good) and differences between these groups were tested using ANOVA.

Poisson regressions with robust errors were used to obtain prevalence ratios (PR) to study the association between plasma tryptophan concentrations and antenatal and postnatal poor sleep quality, and probable depression and anxiety. PRs were calculated because odds ratios are likely to overestimate the association in cross-sectional studies when the outcome of interest is not rare.(Coutinho et al., 2008) Ordered logistic regressions (P parallel test>0.10) were used to study the associations between tryptophan concentrations and the subcomponents of the PSQI, because the subcomponents scores were ordinal (0-3 points). Lastly, to combine the outcomes sleep quality and mental well-being we categorized participants in 3 groups: 1) good sleepers without mental health symptoms, 2) poor sleepers without mental symptoms, 3) poor sleepers with probable mental health symptoms. Participants with good sleep quality and probable mental health symptoms were excluded due to low frequencies (antenatal: anxiety n=35, depression n=3; postnatal anxiety n=12, depression n=0). Two separate robust Poisson regression models were performed having group 1 as the reference. This method was indicated as a valid alternative to multinomial logistic regressions (Camey et al., 2014). A significant difference between groups was calculated with posthoc F-test. The analyses were adjusted for age (years), household income (<S$1999, S$2000-5999, >S$6000), ethnicity (Chinese, Malay, Indian), regular pre-pregnancy smoking (y/n), moderate to intense physical activity (y/n) and log-transformed plasma PLP (µmol/L). Postnatal analyses were additionally adjusted for antenatal measures of sleep or mental well-being.

In sensitivity analyses, we investigated the potential confounding of omega-3 fatty acid concentrations (Judge et al., 2012), and predominantly nighttime feeding (Kant and Graubard, 2014). Moreover, results were stratified by ethnicity to further investigate potential confounding and effect modification (Zhou et al., 2016). Furthermore, when investigating sleep quality, we additionally adjusted for mental well-being (depression or anxiety) (Silber and Schmitt, 2010) and vice versa. Lastly, we analyzed the association between the kynurenine:tryptophan ratio and sleep quality and mental well-being.

Missing values for household income (n=35), pre-pregnancy BMI (n=37), weight gain up to 26-28 weeks gestation (n=39) were multiple imputed for 20 times including the outcome variables, plasma tryptophan, and all potential confounders as covariates in the analyses. Results from the 20 datasets were pooled. Significance was set at P<0.05 and STATA version 14.1 (StataCorp LP, USA) was used for all statistical analyses.

**Results**

*Participant characteristics*

For the antenatal study sample (n=527), the included participants were more likely to be employed, married, or smoking, and had a higher household income, and higher education level as compared to the excluded participants (n=675; **Supplemental table 1**).

Mean plasma tryptophan concentrations were 48.0 µmol/L (SD: 8.09; range: 19.7-75.6). During pregnancy, 329 (57.5%) out of 572 participants were categorized as poor quality sleepers **(Table 1)**. These poor sleepers, as compared to the good sleepers, had lower plasma tryptophan concentrations and plasma kynurenine concentrations, were less likely to be involved in moderate to intensive physical activity, reported later bedtimes, were less likely of Chinese ethnicity, and more likely to suffer from antenatal probable depression and probable anxiety. Participants with antenatal probable anxiety had a trend towards lower plasma tryptophan concentrations as compared to participants without anxiety (Mean difference: 1.33 µmol/L SE: 1.34; P=0.083). No difference in plasma tryptophan concentrations was observed between participants with or without antenatal probable depression (P=0.30), postnatal probable depression (P=0.52), or postnatal probable anxiety (P=0.33).

*Tryptophan concentrations during pregnancy with antenatal sleep quality and mental well-being*

Higher plasma tryptophan concentrations during pregnancy were associated with a lower prevalence of antenatal poor sleep quality in the crude model and when adjusted for covariates [PR: 0.88 (95%CI 0.80, 0.97) per 10 µmol/L; **Table 2**]. When examining the PSQI subcomponents, we observed that higher tryptophan concentrations were associated with lower scores for subjective sleep quality disturbance [OR: 0.76 (95% CI 0.61, 0.95)], habitual sleep efficiency [OR: 0.75 (95% CI 0.59, 0.95)], and sleep disturbances [OR: 0.79 (95% CI 0.63, 0.98)], adjusting for all covariates. Inverse associations between plasma tryptophan and sleep latency and sleep duration were also observed, but these were no longer apparent after including all covariates. No associations were observed between tryptophan concentrations and probable antenatal depression and anxiety.

We additionally investigated whether the coexistence of probable depression or probable anxiety in poor sleepers showed different associations with tryptophan. The association with higher plasma tryptophan was strongest in those having both poor sleep quality and probable anxiety [PR: 0.80 (95% CI 0.67, 0.95) per 10 µmol/L] including all covariates, and weaker in those with just poor sleep quality [PR: 0.90 (95% CI 0.79, 1.02) per 10 µmol/L; **Table 3**). Similarly, the strongest association was observed in those with poor sleep quality and coexisting probable depression [crude PR 0.64 (95% CI 0.44, 0.93) per 10 µmol/L] as compared to those with just poor sleep quality [crude PR: 0.85 (95% CI 0.77, 0.93) per 10 µmol/L]; however after including all covariates the first association attenuated [adjusted PR 0.70 (95% CI 0.45, 1.09) per 10 µmol/L ].

*Tryptophan concentrations during pregnancy with postnatal sleep quality and mental well-being*

No associations were observed between plasma tryptophan concentrations during pregnancy and postnatal subjective sleep quality, probable depression, probable anxiety (**Supplemental table 2**), or the combination of postnatal sleep and mental wellbeing (**Supplemental table 3**).

*Sensitivity analyses*

The findings were similar after additional adjustment for antenatal and postnatal probable depression and probable anxiety, omega-3 fatty acids or predominantly nighttime feeding (data not shown). No effect modification by ethnicity was observed (Pinteraction=0.30). No associations were observed between the kynurenine:tryptophan ratio during pregnancy and antenatal and postnatal poor sleep quality, and mental well-being.

**Discussion**

To our knowledge, this is the first study to report on the association between plasma tryptophan during pregnancy and subjective sleep quality and mental well-being in pregnant women. This is important because sleep and mental well-being are important during pregnancy and can negatively affect both maternal and offspring health. We found that higher plasma tryptophan concentrations measured at 26-28 weeks gestation were associated with a lower risk of antenatal poor sleep quality, and in particular in those with accompanying probable anxiety. Antenatal plasma tryptophan concentrations were not independently associated with probable depression or probable anxiety during the perinatal period. No associations were observed between tryptophan status during pregnancy and subjective sleep or mental well-being assessed three months post-delivery.

*Comparison with the literature*

In general, total plasma tryptophan concentration is lower in pregnant women as compared to non-pregnant (Badawy, 2017), presumably through tryptophan degradation by immune activation as a defense mechanism against fetal rejection (Maes et al., 2001; Maes et al., 2002). The tryptophan concentrations in our study were comparable to those previously reported in pregnant women from 2nd trimester until delivery and varied from 33 to 59 µmol/L (Flachaire et al., 1993; Kamimura et al., 1991; Maes et al., 2002; Nilsen et al., 2012; Schröcksnadel et al., 1996; Schrocksnadel et al., 2003). Furthermore, two studies reported the kynurenine:tryptophan ratio during pregnancy; at 18 weeks gestation (0.019 µmol/µmol),(Nilsen et al., 2012) at 34-37 weeks gestation (0.083 µmol/µmol) (Alegre et al., 2008) and at delivery (0.035 µmol/µmol) (Maes et al., 2002), whereas our ratio measured at 26-28 weeks gestation was considerably higher.

While there are no other observational studies in this area to date, our findings are in line with the conclusion from a review stating that tryptophan supplementation in trials have generally shown to improve sleep quality measures (Silber and Schmitt, 2010). The trials included in this review increased brain tryptophan availability by supplementing with pure tryptophan, serving a high carbohydrates/low protein diet or tryptophan-rich α-lactalbumin protein diet in patients with sleep disturbances or healthy adults. More recent work has further confirmed the positive association between tryptophan and sleep quality in adults (Bravo et al., 2013) and in patients who recovered from depression (Haynes et al., 2004).

We did not observe any independent associations between plasma tryptophan concentrations and perinatal depression or anxiety, which corroborates results from others (Maes et al., 2001; Roomruangwong et al., 2016; Silber and Schmitt, 2010), but not with all (Booij et al., 2002; Shaw et al., 2002a, b). It was suggested that these mixed findings from previous studies might be explained by differing plasma tryptophan concentrations, stemming from different tryptophan treatments, and dose (Silber and Schmitt, 2010). Another explanation could be the inter-individual variation between participants, since some studies showed stronger effects in women or in patients with more depressive episodes, and the associations appeared less strong in patients having treatment with selective-serotonin-reuptake inhibitor (Booij et al., 2002). However, we showed that plasma tryptophan had a stronger association with poor sleep quality in those with coexisting probable anxiety. A review on tryptophan loading and mental well-being and sleep has suggested that tryptophan supplementation was particularly useful in participants experiencing some sleep disturbance and less in healthy participants (Silber and Schmitt, 2010). Moreover, it was shown that 5-HT vulnerable subjects as depicted by 5-HTTLPR genotype and stress-prone individuals especially benefitted from tryptophan augmentation (van Dalfsen and Markus, 2015). The coexisting probable anxiety in our poor sleepers may therefore indicate a higher vulnerability in the serotonin system. Plasma tryptophan was not associated with the combination of having both poor sleep quality and probable depression after including all covariates. This could be explained by our low number of participants with probable depression (n=36).

We observed no associations between plasma tryptophan status during pregnancy and postnatal sleep or mental well-being, possibly due to the lower number of participants with postnatal complete data (n=246) and the subsequent lower power to detect subtle associations. Another possibility might be that plasma tryptophan measured at 26-28 weeks gestation might not be associated with postnatal health, because it has been shown that the concentrations fluctuate over gestation (Maes et al., 2001). Maes et al.(Maes et al., 2002) showed that plasma kynurenine and the kynurenine:tryptophan ratio measured 3-6 days before the anticipated date of delivery was associated with postnatal depression and anxiety symptoms in healthy pregnant women. In contrast, third-trimester plasma tryptophan concentrations were not associated with postnatal EPDS or STAI scores in healthy pregnant women (Roomruangwong et al., 2016).

*Potential mechanisms*

Some mechanisms have been suggested that might explain the association between plasma tryptophan concentrations and poor sleep quality. Tryptophan is involved in brain serotonin availability, which influences sleep latency (Silber and Schmitt, 2010). Furthermore, serotonin is the precursor for the hormone melatonin, which is involved in circadian rhythms including sleep quality (Costello et al., 2014). It has been shown that tryptophan-rich foods or intravenous tryptophan can alter melatonin synthesis (Bravo et al., 2013; Fukushige et al., 2014; Hajak et al., 1991; Peuhkuri et al., 2012).

Another possible mechanism is that plasma tryptophan concentration is a marker for cortisol status. Cortisol concentrations can activate the tryptophan 2,3-doxygenase enzyme, which degrades tryptophan to kynurenine, thereby perhaps lowering the brain tryptophan availability (Oxenkrug et al., 2013). Moreover, higher 24-hour urinary cortisol excretion has been found in poor sleepers or primary insomniacs as compared to controls and urinary cortisol has been associated with total wake time (Roth, 2007). In addition, cortisol levels have been associated with anxiety (Kane et al., 2014), which may explain our more profound results in those having both poor sleep quality and probable anxiety. However, this hypothesis has yet to be explored in pregnancy.

*Strengths and limitations*

A strength of the present study is the relatively large sample size with complete information on plasma tryptophan concentrations, tryptophan metabolites, sleep and mental well-being in pregnant women. However, the prevalence of probable depression and anxiety without poor sleep quality in the subgroup analyses was too low for further analyses, and larger cohorts in pregnant women with higher prevalence of probable depression are warranted. Moreover, we used plasma tryptophan concentrations that represent dietary tryptophan intake and this has the advantage over traditional dietary assessment tools that it contains no recall bias.

Some limitations of the study need to be acknowledged. Plasma tryptophan concentrations, antenatal sleep, and mental well-being were measured at the same time point, meaning that no causal inference can be made. Thus, it may be possible that sleep quality and mental well-being affected dietary intake and consequently tryptophan concentrations (Dashti et al., 2015). Moreover, additional information on free plasma tryptophan or plasma large neutral amino acids concentrations might have provided a better prediction of brain tryptophan availability and subsequently serotonin and melatonin synthesis (Moore et al., 2000; Peuhkuri et al., 2012), but unfortunately these were not assessed. Secondly, sleep and mental mood were assessed subjectively by self-report, which may induced misreporting and thereby attenuating the results. However, all questionnaires have been validated against more objective measures of sleep and mental mood assessments (Backhaus et al., 2002; Buysse et al., 1989; Gibson et al., 2009; Kozinszky and Dudas, 2015; Meades and Ayers, 2011). Thirdly, medication use in our cohort was only ascertained once at 12 weeks gestation and start of psychotropic drugs later than 12 weeks gestation may have introduced misclassification of our participants and thereby attenuated our results. Furthermore, generalizability of our study results is limited to generally healthy Asian pregnant women with a low prevalence of depression. Lastly, like all observational studies we cannot exclude the possibility of residual confounding, although our statistical models did include many covariates known for their association with tryptophan and sleep or mood.

*Conclusions and future studies*

In conclusion, we observed that higher plasma tryptophan concentrations during pregnancy were associated with a 12% lower prevalence of poor sleep quality in an Asian mother-offspring cohort and a 21% lower prevalence in those with poor sleep and probable anxiety. No associations were observed for perinatal mental well-being or postnatal sleep quality. These results might be useful to design future dietary interventions in pregnant women suffering from poor sleep quality, particularly together with anxiety symptoms, as trials have shown to successfully improve insomnia in non-pregnant adults. Lowering poor sleep quality and mental disorders is of vital importance as it reduces the risk for postnatal mental distress and promotes maternal and offspring health. However, our observations should firstly be confirmed in other studies with pregnant participants and the causality of this association should be further explored using prospective studies.

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**Figure 1.** Flowchart of study participants at 26-28 weeks of gestation and at 3 months postnatal.

PSQI: Postnatal Sleep Quality Index, EPDS: Edinburgh Postnatal Depression Scale, STAI: State-Trait Anxiety Inventory

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| **Table 1.** Participants characteristics\* according to sleep quality in 572 participants of the GUSTO cohort |
|  | Poor sleep quality PSQI > 5 | Good sleep quality PSQI ≤ 5  | P-value† |
|  |  n=329 | n=243 |  |
| Tryptophan (µmol/L) | 46.9 (7.9) | 49.4 (8.2) | **<0.001** |
| Kynurenine (µmol/L) | 1.01 (0.19) | 1.06 (0.20) | **<0.001** |
| Kynurenine:Tryptophan ratio | 0.33 (-0.93-1.14) | 0.19 (-0.82-1.20) | 0.467 |
| pyridoxal 5’-phosphate (µmol/L)  | 64.7 (29.9-133.0) | 84.7 (42.6-149.0) | 0.150 |
| Time to go to bed (hh:mm)  | 23:27 (01:23) | 23:02 (01:18) | **<0.001** |
| Time of wake up (hh:mm) | 07:36 (02:08) | 07:23 (01:39) | 0.194 |
| Age (y) | 30.6 (5.2) | 30.7 (4.8) | 0.783 |
| Pre-pregnancy BMI (kg/m2) | 22.7 (4.6) | 22.6 (4.7) | 0.747 |
| Weight gain at 26-28 weeks gestation (kg) | 8.9 (4.4) | 8.3 (5.1) | 0.188 |
| Ethnicity |  |  | **0.008** |
|  Chinese | 47.7 | 60.5 |  |
|  Malay  | 31.6 | 22.2 |  |
|  Indian | 20.7 | 17.3 |  |
| Household income |  |  | 0.512 |
|  <S$1999 | 16.7 | 10.5 |  |
|  S$2000-S$5999 | 54.3 | 60.4 |  |
|  >S$6000 | 29.1 | 29.1 |  |
| Moderate-intensive physical active  | 22.3 | 31.7 | **0.015** |
| Pre-pregnancy smoking regularly  | 15.5 | 10.3 | 0.069 |
| Plasma omega-3 fatty acids (µg/mL) | 134 (98-192) | 138 (101-214) | 0.672 |
| Plasma omega-6 fatty acids (µg/mL) | 766 (607-1010) | 776 (598-1028) | 0.672 |
| Expecting first baby | 46.7 | 42.5 | 0.516 |
| Probable antenatal depression  | 17.9 | 2.9 | **<0.001** |
| Probable antenatal anxiety  | 44.2 | 19.8 | **<0.001** |
| \*values presented are mean (SD), median (IQR) or percentages |
| †P for difference was tested using F-test  |

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| **Table 2.** Associations between plasma tryptophan concentrations (per 10 µmol/L) during pregnancy and antenatal subjective sleep measures and mood in 572 participants of the GUSTO cohort |
|  |  | Crude |  | Multivariate\* |
|  | **Cases** | **PR** | **95% CI** | **P** |  | **PR** | **95% CI** | **P** |
| Poor sleep quality | 329 | 0.85 | 0.77, 0.92 | **<0.001** |  | 0.88 | 0.81, 0.97 | **0.008** |
| Probable depression | 39 | 0.82 | 0.58, 1.16 | 0.260 |  | 0.91 | 0.65, 1.27 | 0.561 |
| Probable anxiety | 150 | 0.86 | 0.72, 1.02 | 0.086 |  | 0.89 | 0.74, 1.06 | 0.185 |
|  |  | **OR**  | **95% CI** | **P** |  | **OR** | **95% CI** | **P** |
| PSQI subcomponents |  |  |  |  |  |  |  |
| Subjective sleep quality | 0.76 | 0.62, 0.94 | **0.011** |  | 0.77 | 0.61, 0.96 | **0.021** |
| Sleep latency | 0.78 | 0.65, 0.94 | **0.008** |  | 0.87 | 0.71, 1.07 | 0.180 |
| Sleep duration | 0.74 | 0.59, 0.93 | **0.010** |  | 0.89 | 0.69, 1.13 | 0.332 |
| Habitual sleep efficiency | 0.71 | 0.57, 0.89 | **0.003** |  | 0.75 | 0.59, 0.95 | **0.019** |
| Sleep disturbances | 0.73 | 0.60, 0.90 | **0.002** |  | 0.79 | 0.64, 0.98 | **0.035** |
| Sleep medication | 0.97 | 0.51, 1.83 | 0.917 |  | 1.11 | 0.57, 2.18 | 0.759 |
| Daytime functioning | 1.01 | 0.83, 1.24 | 0.889 |  | 1.07 | 0.86, 1.32 | 0.534 |
| \*Adjusted for age (years), household income (<S$1999, S$2000-5999, >S$6000), ethnicity (Chinese, Malay, Indian), regular pre-pregnancy smoking (y/n), moderate to intense physical activity (y/n), and plasma PLP concentrations (µmol/L) |

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| **Table 3.** Two separate robust Poisson regression analyses for the association between tryptophan concentrations (per 10 µmol/L) during pregnancy and antenatal subjective sleep quality, and mood in participants of the GUSTO cohort |
|  | Good sleep quality & no anxiety (n=208) |  | Poor sleep quality & no anxiety (n=214) |  | Poor sleep quality & probable anxiety (n=115) |
|  | **PR** | **95% CI** |  | **PR** | **95% CI** | **P** |  | **PR** | **95% CI** | **p** |
| Crude | 1.0 | Ref |  | **0.87** | **0.77, 0.98** | **0.021** |  | **0.73** | **0.61, 0.86** | **<0.001** |
| Model 1 | 1.0 | Ref |  | **0.90** | **0.79, 1.02** | **0.091** |  | **0.79**  | **0.67, 0.94** | **0.008** |
|  | Good sleep & no depression (n=240) |  | Poor sleep & no depression (n=293) |  | Poor sleep & probable depression (n=36) |
|  | **PR** | **95% CI** |  | **PR** | **95% CI** | **P** |  | **PR** | **95% CI** | **p** |
| Crude | 1.0 | Ref |  | 0.85 | 0.77, 0.93 | **0.001** |  | **0.64** | **0.44, 0.93** | **0.020** |
| Model 1 | 1.0 | Ref |  | 0.88 | 0.80, 0.97 | **0.013** |  | 0.70 | 0.45, 1.09 | 0.115 |
| Model 1: adjusted for age (years), household income (<S$1999, S$2000-5999, >S$6000), ethnicity (Chinese, Malay, Indian), regular pre-pregnancy smoking (y/n), moderate to intense physical activity (y/n), and plasma PLP concentrations (µmol/L) |