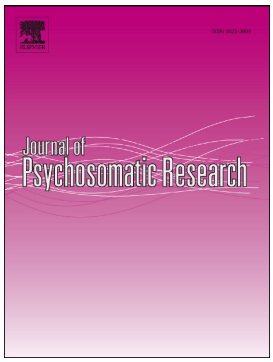

Maternal tiredness and cytokine concentrations in mid-pregnancy

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Maternal Tiredness and Cytokine Concentrations in Mid-Pregnancy

The short running head: Maternal Tiredness and Cytokine Concentrations

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The authors have given their contribution to the present study by participated in designing the study, analyzing the data and revising the manuscript. Dr Kaartinen has taken the main responsibility of writing the manuscript as the first author. All authors have seen and given their final approval for the submitted version to be published. There are no other papers that are based on the same data set, in press, submitted or in preparation.

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Maternal Tiredness and Cytokine Concentrations in Mid-Pregnancy

Abstract

Objective

Sleep disturbances relate to altered levels of inflammatory mediators in general population, but not much is known about the associations between sleep disturbances and inflammatory mediators during pregnancy. The present exploratory study investigated whether insomnia, tiredness, general sleep quality, and insufficient sleep duration during pregnancy relate to the concentrations of maternal peripheral circulating cytokines. As sleep disturbances are frequently observed in mood disorders, the results were controlled for symptoms of depression and anxiety.

Methods

137 participants were randomly drawn from a representative FinnBrain Birth Cohort. Serum concentrations of selected cytokines were analyzed using Multiplex bead arrays from blood samples drawn at the gestational week 24. The sleep disturbances were evaluated using the Basic Nordic Sleep Questionnaire. Depressive and anxiety symptoms were measured with the Edinburgh Postnatal Depression Scale and the anxiety subscale of the self-rated Symptom Checklist 90, respectively.

Results

Enhanced tiredness was associated with cytokine concentrations of IL-2, IL-10, IL-12, IL-13, and TNF-α. The observed associations resembled a reversed U-shaped curve rather than being linear. Having a good general sleep quality was associated with higher logarithmic cytokine concentrations of IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, and IFN-γ. There was no evidence for associations between insomnia or sleep loss and cytokines.

Conclusions

Maternal subjective tiredness and good general sleep quality were associated with altered levels of immunological markers during pregnancy. The association was independent from symptoms of depression and anxiety.

Keywords: Cytokines, Immunology, Pregnancy, Sleep, Sleep Quality, Tiredness

1. Introduction

Sleep disturbances with varying severity are reported by approximately 84% of women during pregnancy (1). Pregnancy-related sleep disturbances include sleep onset insomnia, sleep maintenance insomnia, as well as shortening of sleep duration along pregnancy (2,3). Sleep disordered breathing, especially snoring, is also common (2,3,4). Sleep disturbances in pregnancy may result in sleepiness, a subjective feeling of tiredness associated with increased sleep propensity, during wake hours (5,6). Increased rates of fatigue and sleepiness are frequently observed throughout normally progressing pregnancies (4,6,7). Reportedly, women also take more naps during pregnancy compared to non-pregnant women (3,4).

Cytokines are signaling proteins of the immune system that are stimulated by infections and other types of inflammation, psychological stress, as well as sleep disturbances (8,9,10,11,12,13,14). Cytokines may be further divided into proinflammatory T-helper 1 lymphocyte produced cytokines (Th1 cytokines, such as IFN-γ, IL-6, and IL-12) and anti-inflammatory T helper 2 lymphocyte produced cytokines (Th2 cytokines, such as IL-4 and IL-13), depending on the response they provoke in the body (13,15). Th1 cytokines are typically induced in anti-microbial and autoimmune immune responses, whereas Th2 cytokines counteract the effects of Th1 cytokines and Th2 cytokines also play a dominant role in allergic reactions. However, while the traditional Th1:Th2 dichotomy to classify different cytokines is often used, it is an oversimplified description of cytokine functions (15).

Pregnancy induces changes in maternal cytokine levels, in order to prevent fetus rejection (16). There is a trend for overexpression of Th2 produced anti-inflammatory cytokines in early pregnancy and a shift towards a Th1 produced proinflammatory cytokine profile when the pregnancy proceeds towards parturition (17). For parturition, proinflammatory cytokines play a central role as they increase the production of prostaglandins and matrix-degrading enzymes that are required in the process of inducing labor (17). Higher peripheral concentrations of certain proinflammatory cytokines, e.g. IL-2, IL-6, IL-12, and IFN-γ, have been shown to associate with preterm delivery (10,18,19).

In general, sleep disturbances have been related to altered levels of inflammatory mediators, especially to IL-6, CRP and TNF-α (8,12,14,20,21), although also contradictory findings have been published (22). Knowledge about possible associations between sleep disturbances and cytokine levels in pregnancy is scarce. Diminished overall sleep quality in early and late pregnancy has been related to increased TNF-α and IL-6 concentrations, respectively (10,11). Disturbances in overall sleep quality have also been associated with increased IL-8 in mid-pregnancy, but only among women with African American ethnicity (23). When analyzed in more detail, a prolonged sleep latency has been related to decreased IL-4 and IL-6

concentrations in the 2nd trimester, and a shortened sleep duration during the 2nd and 3rd trimester with enhanced IL-6 production in the 3rd trimester (10,11,23). Among pregnant women with depression, low sleep efficiency (the ratio of the total sleep duration at night compared to the time spent in bed) has been shown to relate to higher IL-6, whereas a greater amount of naps and shorter sleep duration (<7h per night) have been associated with higher TNF-a levels, and with higher IL-8 levels, respectively (24). As depression may alter cytokine levels, these results are not easily comparable with those of non-depressed pregnant women (24,25,26,27,28). However, the association between decreased sleep efficiency and enhanced IL-6 production has also been observed among non-depressed women in late pregnancy (10). Sample sizes in the above-mentioned studies have been rather small, and possible confounding factors, such as body mass index (BMI) and age, have not been routinely controlled for.

Our aim was to conduct an exploratory study to investigate whether insomnia, tiredness, general sleep quality, and insufficient sleep duration during pregnancy are associated with the concentrations of maternal peripheral circulating cytokines. Compared to the previous studies investigating associations between sleep disturbances and cytokines among pregnant women (10,11,23,24), a wider range of pro- and anti-inflammatory cytokines was under interest in the present study. We hypothesized that sleep disturbances, presenting as greater levels of insomnia, tiredness, and sleep loss, and as lessened general sleep quality, would be associated with a proinflammatory immune response. We have previously observed that maternal prenatal symptoms of depression and anxiety are positively associated with peripheral circulating cytokines IL-5, IL-9, and IL-13 in mid-pregnancy (26). Also, as sleep disturbances are frequently observed in depression and anxiety, symptoms of depression and anxiety were controlled for in the present study (29).

2. Methods

2.1. Participants

The study population has been previously described (26) and, hence, only a brief description is given here. The study participants were drawn from a larger population-based pregnancy cohort, the FinnBrain Birth Cohort Study (www.finnbrain.fi) (30), currently ongoing in Southwest Finland. The first consecutive 150 pregnant women participating in the cohort and attending the mid-pregnancy laboratory visit were included as potential subjects in the present study. Exclusion criteria included acute infection (n=4), any chronic inflammatory condition or autoimmune disorder (n=6) and use of oral corticosteroids (n=1). These exclusion criteria data were gained from the participants' self-reports and monitored by the research nurse at the laboratory visit. Further, two subjects were excluded due to missing data in the relevant sleep questionnaires. Thus, after the exclusions, 137 subjects aged from 19 to years 42 were included in the study sample.

The study protocol was approved by the Ethics Committee of Hospital District of Southwest Finland (ETMK 57/180/2011). The subjects gave their written informed consent after they received oral and written information on the study.

2.2. Questionnaires

Data on background and potential confounding variables were collected via self-report questionnaires delivered to the participants at gestational week (gwk) 14 or from the Finnish National Birth Register kept by the National Institute for Health and Welfare (www.thl.fi). These data included information on maternal alcohol consumption (frequency and amount), smoking (current smoking yes/no, the number of cigarettes/day), asthma (yes/no), allergies (yes/no), gestational diabetes (yes/no), and maternal age at delivery. Maternal pre-pregnancy BMI (kg/m^2) was collected from maternity clinic records (missing BMI values were imputed by the study population median value for four subjects). The level of education was defined as low (basic education, 9 years), medium (undergraduate studies, vocational education, 9-12 years), or high (university degree or comparable, over 12 years) (26).

The questionnaires concerning sleep quality and quantity, depressive symptoms and overall anxiety were sent to the participants at gwks 14, 24 and 34, representing the second, and the third trimesters. Responses concurrent with the collection of venous blood samples (gwk 24) were used for the analyses of the present study. Missing data concerning gwk 24 sleep items were imputed for 13 subjects by using their responses on gwk 14 questionnaires. The number of missing sleep items was one or two for ten subjects, and five for one subject. Two subjects did not have any sleep data from gwk 24, and all their sleep data were thus derived from gwk 14 questionnaires. The exclusion of the three subjects whose number of imputed sleep items was five or more did not change the results. Thus, subjects with imputed sleep item data were not excluded from the sample.

The characteristics of sleep and sleep disturbances were evaluated using the Basic Nordic Sleep Questionnaire (BNSQ), which includes 27 sleep related questions (31). In the present study, BNSQ questions concerning insomnia symptoms ([1] difficulty falling asleep, [2] monthly frequency of nocturnal awakenings, [3] daily frequency of nocturnal awakenings, [4] too early morning awakenings, [5] general sleep quality), and tiredness symptoms ([1] morning tiredness, [2] daytime tiredness, [3] napping during the day) were of interest. Insomnia and tiredness items were rated on a five-point-scale ranging from (1) never or less than once a month, 2) less than once a week, 3) 1 – 2 days a week, 4) 3 – 5 days a week, to 5) daily or almost daily during the past month. The insomnia score was computed by calculating the sum score of the following BNSQ items: difficulty falling asleep, monthly frequency of nocturnal awakenings, daily frequency of nocturnal awakenings, too early morning awakenings, and general sleep quality (Cronbach's alpha 0.71). The tiredness score was computed by calculating the sum score of the BNSQ items of morning

tiredness, daytime tiredness, and napping during the day (Cronbach's alpha 0.69). Besides using the insomnia score, general sleep quality was used as an independent insomnia variable in subsequent analyses. General sleep quality was treated as a categorical variable with two categories representing good, and diminished general sleep quality (scale ranges 1-3, and 4-5, respectively). Sleep duration was measured as actual sleep duration, preferred sleep duration, and sleep loss. Average actual sleep duration and preferred sleep duration were inquired by the following BNSQ questions: ”How many hours do you usually sleep per night?” and ”How many hours of sleep do you need per night (how many hours would you sleep if you had the possibility to sleep as long as you need to)?”, respectively, and expressed in hours. For actual sleep duration and preferred sleep duration, the responses given with a range of sleeping hours (e.g. 7 – 8 h) were transformed into their arithmetic means (e.g. 7.5 h). Sleep loss was determined by subtracting the actual sleep duration from preferred sleep duration (hours). For analyses, sleep loss was divided into two subcategories representing sleep loss less than two hours, and sleep loss of at least two hours.

Depressive symptoms were monitored at gwk 24 with the Edinburgh Postnatal Depression Scale (EPDS) (32), that is a self-report questionnaire consisting of 10 items and scored on a 4-point Likert scale (0–3 points/item), total score thus ranging between 0 and 30. The EPDS can also be used to study prenatal depression (33,34).

Symptoms of overall anxiety were assessed at gwk 24 with the anxiety subscale of the self-rated Symptom Checklist 90 (SCL-90-anx) (35,36). The anxiety subscale consists of 10 items scored on a 5-point Likert scale (0–4 points/item), and the range of the total sum score is thus 0–40.

2.4. Cytokine concentrations

The venous blood samples for measuring cytokine levels were drawn on gwk 24 during office hours, i.e. between 8 am and 4 pm. Blood samples were drawn by venipuncture and serum was separated, aliquoted and stored at -70 °C until analyses. The cytokine concentrations were analyzed in a single assay run using Bio-Plex Pro Human Cytokine 21- and 27-Plex Assay kits (Bio-Rad, Hercules, CA). The amount of beads, detection antibodies and streptavidin-phycoerythrin conjugate were used at half of their recommended concentrations, as previously described (26,37,38,39,40,41).

In our previous study, associations between maternal prenatal depressive and anxiety symptoms and concentrations of the cytokines IL-4, IL-5, IL-6, IL-9, IL-10, IL-12, IL-13, TNF-α, and interferon gamma (IFN-γ), were investigated (26). As depression and anxiety are associated with sleep disturbances, the aim in the present study was to investigate if maternal sleep characteristics had independent associations with these cytokine concentrations during pregnancy (29). Along with the aforementioned cytokines, the proinflammatory cytokines IL-1β, IL-2 and IL-8 were additionally investigated, as they have been

previously reported to have associations with pregnancy complications and sleep problems or disorders (8,15,24). IFN-γ/IL-4 ratio was calculated to illustrate a rough estimate of the Th1/Th2 balance (42).

2.5 Statistical analyses

2.5.1. Depressive and anxiety symptoms

As the study participants were recruited from the general population, where the frequency of possible clinical depression was expectedly low, a continuous total sum score of the Edinburgh Postnatal Depression Scale (EPDS) (32) was used as a measure of depressive symptoms in the main analyses. For the same reason, a continuous sum score of the SCL-90 anxiety subscale (SCL-90-anx) (35,36) was used to measure the level of anxiety among the participants.

2.5.2. Cytokines

Logarithmic transformations of measured cytokine levels were used in the analyses to reduce skewness of distributions.

2.5.3. Associations between cytokines and the sleep variables

Standard linear regression analysis was used to study associations between the cytokines and the sleep variables. First, regression analysis was conducted separately for every cytokine under interest by using (the natural logarithm of) the cytokine concentration as the dependent variable, and the tiredness score, the insomnia score, the dichotomous sleep loss and the selected covariates (BMI, age, depressive and anxiety symptoms) as independent variables. Associations between the tiredness score and each cytokine were modeled by natural cubic splines (43) with two degrees of freedom to be able to model the non-linear associations between them (see Figure 1). To investigate possible associations between cytokines and general sleep quality, similar regression analysis was conducted for every cytokine under interest by using the dichotomous general sleep quality and the selected covariates as independent variables. Covariate selection was conducted according to the observed associations between potential covariates (depressive symptoms, symptoms of anxiety, age, and BMI) and cytokines in our previous study (26). Additionally, in a large-scale population study IL-2, IL-5, IL-10, IL-12, IL-13, and TNF-α have been associated with BMI among non-pregnant women (40). Additionally, age is linked with some peripheral cytokine profiles (26,44). As the present study is among the first to investigate a larger set of cytokines in the context of maternal prenatal sleep characteristics, these analyses were considered explorative and the significance level was set at the p value of 0.05. All the analyses were made using R (45) with the packages ggplot2 (46) for Figure 1 and splines (45) for fitting natural cubic spline curves.

3. Results

Description of the study population

The study population characteristics and descriptive statistics of the sleep and cytokine variables are presented in Tables 1, 2, and 3, respectively.

- insert Table 1 about here –

- insert Table 2 about here–

- insert Table 3 about here–

Insomnia

In the regression analyses, the insomnia score was not associated with any logarithmic cytokine concentration (Table 4).

Sleep quality

In all, 121 (88.3%) women reported having good, and 16 (11.7%) women diminished general sleep quality. Having a good general sleep quality was associated with higher logarithmic cytokine concentrations of IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, and IFN-γ (Table 5).

Tiredness

The tiredness score was associated with cytokine concentrations of IL-2, IL-10, IL-12, IL-13, and TNF-α (Table 4). As seen in Figure 1, the observed associations between the tiredness score and logarithmic cytokine concentrations resembled a reversed U-shaped curve rather than linear (Figure 1).

Sleep loss

105 (76.6%) women had sleep loss less than two hours, and 32 (23.4%) women more than two hours. The amount of sleep loss did not relate to cytokine concentrations (Table 4).

Covariates

In the models where the three sleep score variables (insomnia score, tiredness score, sleep loss) were entered simultaneously, no associations between IL-2, IL-10, IL-12, IL-13, or TNF-α logarithmic cytokine concentrations and the selected covariates, i.e. depressive symptoms (p=0.074 – 0.95), anxiety symptoms (p=0.45 – 0.82), age (p=0.078 – 0.50), or BMI (p=0.071 – 0.67), were observed.

When analyzing the associations between general sleep quality and the cytokine concentrations, age was related to IL-10 (p=0.019), and IL-12 (p=0.031), and BMI to IL-12 (p=0.050), and IL-13 (p=0.030).

- insert Table 4 about here –

- insert Figure 1 about here –

- insert Table 5 about here –

4. Discussion

Our hypothesis of an association between maternal prenatal sleep disturbances and peripheral pro-inflammatory cytokine concentrations was supported partially. Both tiredness and the general sleep quality had associations with IL-2, IL-10, IL-12, and IL-13. Additionally, tiredness was related to TNF-α and the general sleep quality was associated with IL-4, IL-6, and IFN-γ concentrations. To our best knowledge, this is the first time when associations between maternal prenatal tiredness and these cytokines have been reported.

There are earlier findings on the association between poor sleep quality and enhanced inflammatory processes in all phases of pregnancy (10,11,23). Conflicting these findings, good general sleep quality was related to heightened IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, and IFN-γ cytokine levels in mid-pregnancy in the present study. Discrepancy between the results could be at least partly explained by differences in methodology. In the previous studies, overall sleep quality has been measured by a total score in the Pittsburgh Sleep Quality Index (PSQI), which includes variety of sleep disturbance items, while in the present study general quality of sleep was measured as a subjective estimation of sleep quality (10,11,23). There is an earlier study showing that compromised subjective quality of sleep relates to enhanced IL-6 production while stimulated with Escherichia coli endotoxin (10). According to the results of the present study, positive associations between good subjective sleep quality and cytokines might also be present without any stimulation and concern wider range of cytokines that earlier observed.

Previous studies have also yielded scattered evidence that also prolonged sleep latency, poor sleep continuity, and shortened sleep duration relate to altered levels of immunological responses during pregnancy (10,11,23,24). However, insomnia or sleep loss were not associated with the level of peripheral cytokines in the present study, where depressive symptoms, the level of anxiety, BMI, and age were controlled for. This implies that similarly as among non-pregnant women, also during pregnancy disturbed sleep in general may not relate to altered immunological responses (47). Different types of sleep disturbances may represent distinctive phenotypes that correspond with differential immunological mechanisms, and for example, may rather relate to a disturbed Th1/Th2 balance, than to isolated altered

cytokine concentrations (48). A mediating agent in these associations might be melatonin that, –besides to the sleep-wake rhythm-, is shown also to regulate immunological processes on a larger scale (49).

Approximately one-fourth of pregnant women report sleepiness in mid- and late pregnancy (6). While the frequency of sleep disturbances increases as the pregnancy proceeds, the frequency of sleepiness seems to remain stable over mid- and late pregnancy (6). Thus, it is possible that sleepiness in pregnancy may not totally result from disrupted sleep (6). Inflammatory mechanisms, especially cytokine IL-6, have been related –in non-pregnant populations- to daytime fatigue in the absence and the presence of medical conditions, e.g. multiple sclerosis and cancer (50,51,52,53). According to the present study, inflammatory mechanisms may relate to increased tiredness or subjective tiredness also in pregnancy. As pregnant women experience more tiredness than non-pregnant women, our notion that the increased tiredness even in this population is associated with a distinct peripheral inflammatory marker pattern, is interesting and potentially important (5,6).

Even though cytokines relate strongly and widely to each other and to CRP, a multivariate model showed that they rather function as independent risk factors than relate to tiredness and good general sleep quality as a solid group (40). The role of cytokines IL-1 and TNF-alfa as sleep promotors has been well documented (54,55). Evidence also suggests that other cytokines regulate the sleep-wake rhythm (54). For an example, whereas IL-1, IL-2, IL-6, and IL-8 promote sleep, IL-4, IL-10, and IL-13 seem to reduce sleep by inhibiting the production of sleep promoting cytokines (54). However, the roles of individual cytokines in sleep regulation seem to be complex, as in certain concentration one cytokine may function as a sleep promoter and in another as a sleep inhibitor (54,55). Interestingly, the observed associations between peripheral cytokines and tiredness were not linear, but rather resembled a reversed U-shaped curve. An earlier observation has also shown that the association between IL-1 and sleep propensity might resemble a reversed U-shaped curve; i.e. whereas in general IL-1 is a sleep inductor, in high doses it becomes a sleep inhibitor (55). It is possible that a negative feedback-loop regulates the level of circulating cytokines by decreasing production of previously overproduced peripheral cytokines in case of prolonged Th1 and Th2 cell responses (56). Also, prolonged or frequent tiredness may induce or result from chronic stress that –via for instance cortisol dysfunction- has potential to produce inflammatory responses that are distinct from inflammatory responses related to short exposure to stressful events (57). Although the mechanisms behind the observed U-shaped association between tiredness and peripheral cytokines were out of the scope of the present study, to our knowledge this is a novel finding regarding a range of cytokines, and warrants further investigation.

In non-pregnant populations, disrupted sleep is related to various negative health consequences, such as increased risk for diabetes, elevated blood pressure and obesity (58,59). Growing evidence suggests that also maternal prenatal sleep disturbances may indicate an increased risk for pregnancy and delivery

complications. Insomnia, shortened sleep duration and sleep deprivation, sleepiness, and decreased sleep quality are reportedly associated, for example, with preterm labor and preeclampsia, as well as longer delivery and operative deliveries, especially caesarean sections (23,60,61,62,63,64,65,66,67,68). Possible mechanisms that mediate the above-mentioned risks may include elevated levels of stress hormones, inflammatory parameters and cytokines, that have been observed among individuals with sleep disturbances (12,13,22,23,66,67,69). In our population, multivariable analyses showed that maternal subjective tiredness and good general sleep quality explained certain cytokine levels after controlling for the effects of depressive and anxiety symptoms. However, the present study does not provide any information on the potential causality of the relationships.

Strengths and limitations

Our study was merited by a randomly selected sample representing a generally healthy female population. As the population was relatively healthy in terms of psychiatric symptoms, extension of our findings to psychiatric populations needs further research. The sample size in the present study is larger than in previous studies among pregnant women. However, even larger samples with repeated assessments would further strengthen power of the study. The present study should also be considered as exploratory, and future studies are needed to investigate associations between cytokines and sleep disturbances in pregnancy. Immunological responses relating to sleep disturbances seem to vary according to the phase of pregnancy, and between women with different ethnicities (10,11,23,24). In addition, cortisol levels were not measured from the participants. This should be considered as a limitation as cortisol is an important modulator of both, the immune system, as well as the sleep-wake rhythm (54,57).

Another strength of this study was the broader range of cytokines compared to previous studies. However, we studied only a single cytokine sample at one time point during the second trimester, which precludes any conclusions on causality or the sequence of the phenomena, and may hinder comparisons to other studies (11). The blood samples were taken during office hours and detailed data on the timing of individual samples was not available. This can be considered as a limitation, as some cytokines are secreted with diurnal fluctuations (70).

The questionnaire we used, the BNSQ, is validated and widely used in different populations (31). It contains a wide panel of sleep items and therefore provides precise information on sleep disturbances and tiredness. Further studies using objective measures might give new perspectives into the understanding of the connections between sleep and inflammation. Previously, we reported that cigarette smoking was unrelated to cytokine concentrations in this population of pregnant women with a very low number of cigarette smokers and a low number of cigarettes consumed/day (26) and thus our results were not controlled for smoking. This precludes the extension of the results to more frequently smoking populations.

Conclusions

Maternal subjective tiredness and good general sleep quality were associated with altered levels of immunological markers during pregnancy, the association being independent from symptoms of depression and anxiety. Based on earlier research, cytokine profiles might affect the course of pregnancy and delivery, as well as offspring health outcomes, which underlines the importance of further research in this area (61,63,66,67,71). While optimal sleep patterns during pregnancy are not known and probably vary between women, subjective tiredness and general sleep quality may present important indicators of a risk of altered inflammatory responses during pregnancy (61).

Conflict of Interest

Miia Kaartinen has received financial support from the Medical Research Fund of Tampere University Hospital. Miia Kaartinen has no other competing interests to report.

Linnea Karlsson has received funding for this research from Signe and Ane Gyllenberg Foundation, Brain and Behavior Research Foundation NARSAD YI Grant #1956, and the Academy of Finland #308589. Linnea Karlsson has no other competing interests to report.

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Mikael Maksimow has no competing interests to report.

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Figure Titles and Captions

Figure 1. Associations between cytokine concentrations and the tiredness score (95% (pointwise) confidence bands marked with grey colour).

Table 1. Characteristics of the study population (n=137).

	Mean (SD)	Range

	or n (%)	
Age (years)	30.6 (4.8)	19-42
BMI (kg/m^2)	25.2 (4.8)	18-46
Parity		
Nulliparous	69 (50.4)	
Multiparous	68 (49.6)	
Education		
Low level	50 (36.5)	
Middle level	36 (26.3)	
High Level	51 (37.2)	
Marital status [1]		
Married/cohabitating	131 (98.5)	
Divorced	0 (0.0)	
Single	2 (1.5)	
Widowed	0 (0.0)	
Asthma	9 (6.6)	
Allergy	57 (41.6)	
Gestational diabetes	28 (20.4)	
Smoking during pregnancy		
Non-smokers	122 (89.1)	
Smokers	15 (10.9)	
Alcohol consumption during pregnancy		
Non-users	112 (81.8)	
Any use	25 (18.2)	
EPDS score	4.38 (3.56)	0-16
SCL-90/anxiety score	2.96 (3.09)	0-15

[1] Four missing values

EPDS= the Edinburgh Postnatal Depression Scale

SCL = Symptom Checklist 90

Table 2. Characteristics of sleep (BNSQ= the Basic Nordic Sleep Questionnaire) (n=137).

	Mean (SD)	Range
Insomnia score	13.0 (3.3)	5 - 23
Difficulty falling asleep	1.8 (0.8)	1 - 4
Monthly frequency of nocturnal awakenings	4.4 (1.0)	1 - 5
Daily frequency of nocturnal awakenings	2.7 (0.9)	1 - 5
Too early morning awakenings	2.0 (1.0)	1 - 5
General sleep quality	2.1 (1.0)	1 - 5
Tiredness score	7.5 (2.6)	3 - 15
Morning tiredness	2.6 (1.2)	1 - 5
Daytime tiredness	2.6 (1.1)	1 - 5
Napping during the day	2.3 (1.1)	1 - 5
Sleep Duration		
Actual length of sleep (hours)	7.8 (0.9)	6 - 11
Preferred duration of sleep (hours)	8.9 (1.1)	6 - 13
Sleep loss	1.1 (1.2)	-2 - 5

Table 3. Cytokine concentrations (pg/mL) (n=137).

	Mean (SD)[1]	Range	Median	Q_1/Q_3
IL-1β	1.7 (0.2)	3.1 - 15.8	5.3	4.7 / 6.3
IL-2	3.0 (0.3)	7.9 - 74.5	20.8	17.5 / 23.3
IL-4	2.5 (0.1)	7.4 - 18.7	13.0	11.6 / 14.3
IL-5	1.8 (0.3)	3.2 - 16.4	5.8	4.8 / 6.9
IL-6	2.7 (0.3)	7.5 - 80.0	14.0	11.7 / 16.4
IL-8	3.5 (0.3)	19.0 - 169.7	30.6	27.6 / 35.6
IL-9	4.2 (0.7)	28.5 - 9213.3	59.2	48.3 / 79.5
IL-10	2.1 (0.5)	2.0 - 33.6	7.5	6.0 / 10.6
IL-12	3.3 (0.6)	9.6 - 249.5	23.7	19.9 / 34.5
IL-13	2.7 (0.4)	6.4 - 46.9	14.2	11.3 / 17.8
TNF-α	4.2 (0.4)	31.8 - 382.1	62.5	56.2 / 76.4
IFN-γ	5.6 (0.2)	148.2 - 609.2	258.3	230.4 / 295.6
IFN-γ/IL-4	20.7 (2.9)	6.1 x 10^6 - 2.6 x 10^15	8.0 x 10^8	1.9 x 10^8 / 2.6 x 10^9

[1]The means and standard deviations are calculated for logarithm transformed values, others from original values.

Table 4. Results of regression analyses examining the associations between the selected sleep variables and cytokines (n = 137). All models included all three sleep parameters (insomnia score, tiredness score, and sleep loss).

	B	*SE B*	t_{128} * / $F_{2, 128}$ **	*p*
IL-1β				
Insomnia score	0.001	0.008	0.18 *	0.86
Tiredness score (2 df) [1]			2.84 **	0.062
Sleep loss (Ref = 'No')	-0.084	0.057	-1.47 *	0.14
IL-2				
Insomnia score	-0.012	0.009	-1.41 *	0.16
Tiredness score (2 df) [1]			4.86 **	0.009
Sleep loss (Ref = 'No')	-0.058	0.066	-0.87 *	0.39
IL-4				
Insomnia score	-0.006	0.004	-1.29 *	0.20
Tiredness score (2 df) [1]			2.77 **	0.067
Sleep loss (Ref = 'No')	-0.030	0.034	-0.87 *	0.39
IL-5				
Insomnia score	-0.003	0.009	-0.30 *	0.76
Tiredness score (2 df) [1]			1.99 **	0.14
Sleep loss (Ref = 'No')	-0.103	0.066	-1.56 *	0.12
IL-6				
Insomnia score	-0.018	0.010	-1.70 *	0.091
Tiredness score (2 df) [1]			2.55 **	0.082
Sleep loss (Ref = 'No')	-0.121	0.079	-1.53 *	0.13
IL-8				
Insomnia score	-0.010	0.009	-1.06 *	0.29
Tiredness score (2 df) [1]			1.96 **	0.15
Sleep loss (Ref = 'No')	-0.077	0.069	-1.11 *	0.27
IL-9				
Insomnia score	-0.020	0.023	-0.86 *	0.39
Tiredness score (2 df) [1]			0.73 **	0.48
Sleep loss (Ref = 'No')	-0.214	0.174	-1.23 *	0.22
IL-10				
Insomnia score	-0.006	0.014	-0.44 *	0.66
Tiredness score (2 df) [1]			4.91 **	0.009
Sleep loss (Ref = 'No')	-0.117	0.105	-1.11 *	0.27
IL-12				
Insomnia score	-0.026	0.016	-1.61 *	0.11
Tiredness score (2 df) [1]			4.00 **	0.021
Sleep loss (Ref = 'No')	-0.124	0.121	-1.02 *	0.31
IL-13				
Insomnia score	-0.013	0.010	-1.28 *	0.20
Tiredness score (2 df)			4.89 **	0.009
Sleep loss (Ref = 'No')	-0.110	0.079	-1.41 *	0.16
TNF-α				
Insomnia score	-0.020	0.012	-1.71 *	0.090
Tiredness score (2 df) [1]			3.35 **	0.038
Sleep loss (Ref = 'No')	-0.150	0.089	-1.68 *	0.095
IFN-γ				
Insomnia score	-0.007	0.006	-1.11 *	0.27
Tiredness score (2 df) [1]			2.37 **	0.098
Sleep loss (Ref = 'No')	-0.079	0.048	-1.64 *	0.10
IFN-γ/IL-4				
Insomnia score	-0.039	0.088	-0.44 *	0.66
Tiredness score (2 df) [1]			0.41 **	0.67
Sleep loss (Ref = 'No')	-1.063	0.664	-1.60 *	0.11

B = Unstandardized regression coefficient. For Sleep loss: the estimated difference in the (average) log-transformed cortisol levels between the groups with sleep loss (>= 2h) and with no sleep loss (< 2h). For Insomnia: The effect of one unit change in the insomnia score on the average log-transformed cortisol level.

SE B = Standard error (of the regression coefficient)

t_{128} = t statistic (with 128 degrees of freedom)

$F_{2, 128}$ = F statistic (with 2 and 128 degrees of freedom)

All models were adjusted for maternal symptoms of depression (EPDS), anxiety (SCL-90/anxiety), age, and pre-pregnancy body mass index (BMI).

[1] The result from comparing the model with and without the two spline terms modeling the non-linear relationship between the Tiredness score and (average) log-transformed cytokine levels.

Table 5. Results of regression analyses examining the associations between the general sleep quality and cytokines (n = 137).

	General sleep quality (Ref = ´Diminished`)			
	B	*SE B*	t_{128}	*p*
IL-1β	0.079	0.070	1.13	0.26
IL-2	0.167	0.081	2.06	0.041
IL-4	0.103	0.041	2.51	0.013
IL-5	0.133	0.080	1.65	0.10
IL-6	0.209	0.097	2.16	0.033
IL-8	0.153	0.084	1.83	0.070
IL-9	0.406	0.210	1.94	0.055
IL-10	0.257	0.129	2.00	0.048
IL-12	0.392	0.148	2.66	0.009
IL-13	0.292	0.095	3.07	0.003
TNF-α	0.159	0.111	1.44	0.15
IFN-γ	0.129	0.059	2.18	0.031
IFN-γ/IL-4	0.721	0.804	0.90	0.37

B = Unstandardized regression coefficient. The estimated difference in the (average) log-transformed cortisol levels between the groups good and diminished sleep quality.

SE B = Standard error (of the regression coefficient)

t_{128} = t statistic (with 128 degrees of freedom)

All models were adjusted for maternal symptoms of depression (EPDS), anxiety (SCL-90/anxiety), age, and pre-pregnancy body mass index (BMI).

Highlights

- Subjective tiredness is related to altered levels of cytokines in pregnancy.
- Good general sleep quality is associated with higher cytokine levels.
- There was no evidence for associations between insomnia or sleep loss and cytokines.

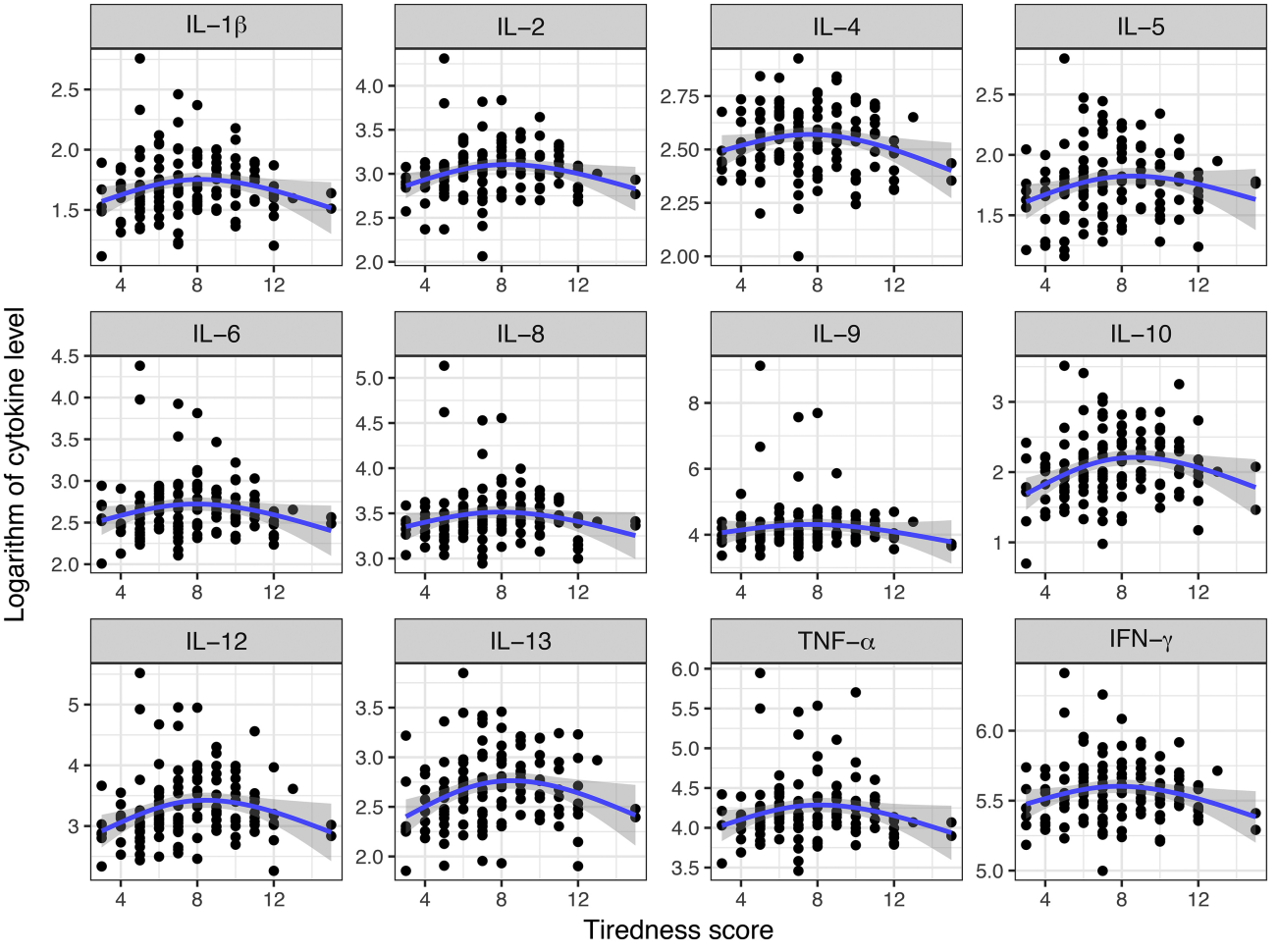
IL−1β
IL−2
IL−4
IL−5
IL−6
IL−8
IL−9
IL−10
IL−12
IL−13
TNF−α
IFN−γ
Logarithm of cytokine level
Tiredness score

Figure 1