



Research Paper

Paracetamol Medication During Pregnancy: Insights on Intake Frequencies, Dosages and Effects on Hematopoietic Stem Cell Populations in Cord Blood From a Longitudinal Prospective Pregnancy Cohort



Lars Bremer^{a,1}, Janina Goletzke^{b,1}, Christian Wiessner^c, Mirja Pagenkemper^b, Christina Gehbauer^d, Heiko Becher^c, Eva Tolosa^d, Kurt Hecher^b, Petra C. Arck^b, Anke Diemert^{b,2}, Gisa Tiegs^{a,*,2}

^a Institute of Experimental Immunology and Hepatology, University Medical Center Hamburg Eppendorf, Martinistrasse 52, Hamburg 20246, Germany

^b Department of Obstetrics and Fetal Medicine, University Medical Center Hamburg Eppendorf, Martinistrasse 52, Hamburg 20246, Germany

^c Institute of Medical Biometry and Epidemiology, University Medical Center Hamburg Eppendorf, Martinistrasse 52, Hamburg 20246, Germany

^d Department of Immunology, University Medical Center Hamburg Eppendorf, Martinistrasse 52, Hamburg 20246, Germany

ARTICLE INFO

Article history:

Received 12 September 2017

Received in revised form 16 October 2017

Accepted 23 October 2017

Available online 9 November 2017

Keywords:

Acetaminophen

Childhood asthma

Hematopoietic stem cells

Lineage development

Pregnancy

ABSTRACT

Background: Paracetamol is the first choice for antipyretic or analgesic treatment throughout pregnancy. Products with Paracetamol are readily available over the counter and therefore easily accessible for self-medication. Epidemiological data on Paracetamol intake pattern during pregnancy and its potential immunological effects are sparse. We aimed to analyze a possible association between Paracetamol medication and numbers of hematopoietic stem cells (HSC) in cord blood.

Methods: The objective was addressed in the PRINCE (PRENATAL DETERMINANTS OF CHILDREN'S HEALTH) study, a population-based prospective pregnancy cohort study initiated in 2011 at the University Medical Center in Hamburg, Germany. 518 healthy pregnant women with singleton pregnancies were recruited during the first trimester. Three examinations were scheduled at the end of the 1st (gestational week 12–14), the 2nd (gestational week 22–24) and the 3rd trimester (gestational week 34–36). For 146 of these women, cord blood flow cytometry data were available. Paracetamol intake was assessed for each trimester of pregnancy.

Findings: Among the 518 enrolled women, 40% took Paracetamol as main analgesic treatment during pregnancy. The intake frequency and dosage of Paracetamol varied between the women and was overall low with a tendency towards higher frequencies and higher dosages in the third trimester. Paracetamol intake, particularly during the third trimester, resulted in decreased relative numbers of HSCs in cord blood, independent of maternal age, first-trimester BMI, parity, gestational age and birth weight (-0.286 (95% CI $-0.592, 0.021$), $p = 0.068$).

Interpretation: Prenatal Paracetamol intake, especially during the third trimester, may be causally involved in decreasing HSCs in cord blood.

© 2017 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

While fever and pain are debilitating and endangering, especially during pregnancy, medication might evoke long-term side effects in the offspring. Although a multitude of fever and pain relievers is available, only few are considered to be safe during pregnancy. Since 1893, *N*-acetyl-*p*-aminophenol (Paracetamol, Tylenol, APAP) became the first-line and widely used medication for pain and fever (Brune et al., 2015). Paracetamol is an over the counter (OTC) drug and sold under different brand names, pure or in combination with vitamin C or caffeine. Paracetamol is apparently safe at therapeutic doses and neither an opioid nor an inhibitor of coagulation. Nevertheless, Paracetamol is able to pass the placenta freely, thus exerting a direct effect on the fetus. Several retrospective and cohort studies addressed the question whether the intake of Paracetamol during pregnancy affects pregnancy

Abbreviations: FACS, fluorescence-activated cell sorting; gw, gestational week; HSC, hematopoietic stem cells; OTC, over the counter; PRINCE, PRENATAL DETERMINANTS OF CHILDREN'S HEALTH.

* Corresponding author at: Universitätsklinikum Hamburg Eppendorf, Institut für Experimentelle Immunologie und Hepatologie, Martinistrasse 52, N27, 20246 Hamburg, Germany.

E-mail addresses: La.Bremer@uke.de (L. Bremer), J.Goletzke@uke.de (J. Goletzke), C.Wiessner@uke.de (C. Wiessner), M.Pagenkemper@uke.de (M. Pagenkemper), C.Gehbauer@uke.de (C. Gehbauer), H.Becher@uke.de (H. Becher), E.Tolosa@uke.de (E. Tolosa), K.Hecher@uke.de (K. Hecher), P.Arck@uke.de (P.C. Arck), A.Diemert@uke.de (A. Diemert), G.Tiegs@uke.de (G. Tiegs).

¹ Contributed equally to this work.

² Shared supervision.

outcome or children's health. Here, the impact of prenatal Paracetamol on neuronal development and children's behavior is currently intensively discussed (Stergiakouli et al., 2016; Saunders and Habgood, 2017; Damkier et al., 2017). Moreover, a Danish cohort study from 2008 revealed a positive correlation between prenatal Paracetamol exposure and children's asthma (Rebordosa et al., 2008). These findings are supported by the work of Shaheen et al., linking Paracetamol to antioxidant gene polymorphisms and wheezing when taken in late pregnancy (Shaheen et al., 2002, 2010). Recently, Magnus et al. published a remarkable Child Cohort Study which demonstrated that pre- and postnatal exposure to Paracetamol is associated with asthma development (Magnus et al., 2016). A meta-analysis revealed that Paracetamol intake during the first trimester seems to elevate the risk of childhood asthma (Cheelo et al., 2015). Nevertheless, association between Paracetamol and risk of childhood asthma varies between the studies and lacks insights on causality, e.g. an alteration of immune ontogeny in response to Paracetamol (Cheelo et al., 2015). Compelling evidence from mouse models showed a reduction of fetal liver HSCs, which could be associated with an increased risk of airway inflammation in the offspring (Karimi et al., 2015; Thiele et al., 2015).

2. Aim

The aim of our present study was to determine the intake pattern of Paracetamol during pregnancy in a longitudinal study. Furthermore, we aimed to investigate possible effects and critical periods of Paracetamol intake on fetal immune ontogeny by analyzing the association between Paracetamol and HSC frequencies in cord blood.

3. Material and Methods

3.1. Study Design and Population

The PRINCE study is conducted at the University Medical Center Hamburg-Eppendorf (UKE) and was initiated in 2011. Inclusion criteria were maternal age of 18 years or higher and a viable singleton pregnancy at gestational week 12–14. Women with chronic infections (HIV, hepatitis B/C), known substance abuse, who were smoking, had multiple pregnancies or pregnancies conceived after assisted reproductive technologies were excluded. Pregnant women were invited to three antenatal visits, once per trimester (gestational weeks 12 to 14, 24 to 26, and 34 to 36). Data on the assessment of relevant covariables is described in detail elsewhere (Diemert et al., 2017). All study subjects signed informed consent forms and the study protocol was approved by the ethics committee of the Hamburg Chamber of Physicians (PV3694).

At the time of analyses, the PRINCE study sample consisted of 620 women. To be included in the present analysis, data on analgesic intake had to be available for each trimester, which was the case for 518 women. For analyses, only those women with no analgesic intake at all were compared to those with Paracetamol medication. Hence, women relying only on other analgesic medication such as ibuprofen were excluded from further analyses (final $n = 483$). Cord blood could be obtained from women delivering at the UKE (30% overall) and analyzed for hematopoietic stem cell ($n = 146$) (Fig. 1).

3.2. Assessment of Analgesic Medication

At each study visit, medication intake was assessed via a questionnaire assisted interview from the study gynecologists. Women were asked to reflect in detail which analgesic they took since the beginning of their pregnancy or the last study visit, respectively. For each analgesic taken, information was asked regarding the brand name, specific date(s) of intake, intake duration and dosage. For the present analyses, total intake dosage as well as duration of intake were summed up for each trimester as well as for the entire pregnancy. Furthermore,

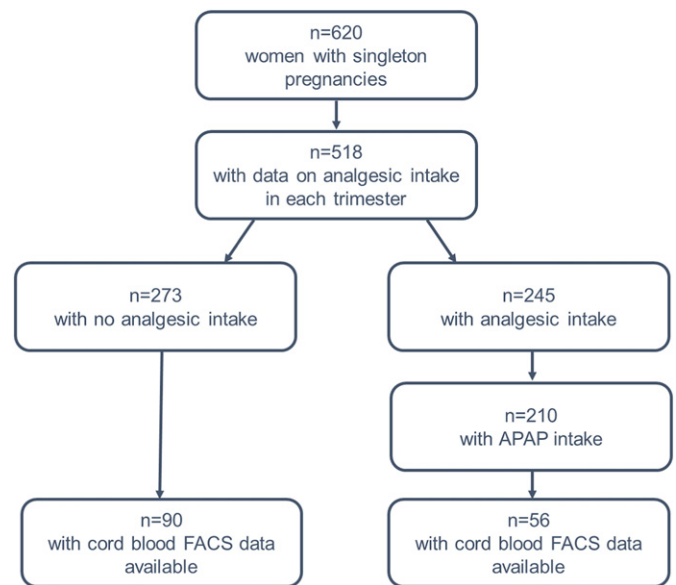


Fig. 1. Flow chart of the study sample. Abbreviations used: FACS, fluorescence-activated cell sorting.

categorical variables were computed: Based on the standard concentrations (500 mg Paracetamol/tablet) available in Germany, women were divided into four a priori defined groups according to Paracetamol intake: ≤ 500 mg, >500 –1500 mg, >1500 –4000 mg, and >4000 mg per day. Regarding intake duration, women were divided into groups of onetime, occasionally (irregular acetaminophen intake), weekly (regular intake at least for two weeks per trimester), and daily (regular intake at least for three continuous days per trimester).

3.3. Cord Blood Analyses

If the woman gave birth at the UKE and signed consent, cord blood was collected. Samples were processed within 24 h after delivery, and 50 μ l whole blood were stained with an antibody mixture as described elsewhere (Diemert et al., 2016). Total HSCs were identified using two independent gating strategies in the CD45-positive mononuclear cell subset (mother population) using CD34 and CD133 as markers on cells with intermediate CD45 expression (CD45int). HSCs are defined as CD45int CD34⁺ cells, while “total HSCs” encompass in addition to the CD34⁺ cells the “early HSC”, defined as the CD34⁻ CD133⁺ (Supplemental Fig. 1).

3.4. Statistical Analyses

Descriptive statistics were used to present analgesic intake in the cohort: Group comparisons were made using *t*-test or ANOVA for normally distributed continuous variables, Kruskal-Wallis test for not normally distributed continuous variables and Chi-square test for categorical variables.

In a first step assessing the association between Paracetamol intake and HSCs, binary variables (Paracetamol intake in pregnancy yes/no) were analyzed using univariate regression models. As variables for HSC populations were not normally distributed, they were logarithmized prior to analyses. If an association was indicated in this first analysis by a statistical trend, further analyses were run to investigate whether a certain period during pregnancy was of particular importance. To achieve this, the structured approach to hypotheses involving binary exposures over the life course, presented by Smith et al. (2015), was used: As we aimed to assess the association between repeatedly measured Paracetamol intake over the course of pregnancy and cord blood HSC populations, applying our research question to

this approach, the two following hypotheses were investigated: the accumulated effect (variable values 0, 1, 2, 3, encoding Paracetamol intake in none, one, two, or each trimester), and critical periods (Paracetamol intake yes/no in each trimester). By using least angle regression, the most relevant hypothesis was identified and, as suggested by Smith et al. (2015) an elbow plot was further used to identify all possible hypotheses of relevance. After identifying the relevant hypotheses, in a third step, univariate and multivariable regression analyses were run with each of the variables encoding the relevant hypotheses. Covariates considered as potentially affecting the association between Paracetamol intake and cord blood immune populations were maternal age, maternal first-trimester BMI, maternal educational status, maternal asthma, allergies, chronic disease, antibiotics medication (yes/no) pregnancy complications (yes/no, questionnaire based), parity ($0 \geq 1$), birth weight, gestational age, and fetal gender. The final model including the relevant covariables conformed to the assumptions of linear regression models (linearity, normality and homoscedasticity of residuals, absence of multicollinearity).

All analyses were carried out by using SAS software (version 9.4; SAS Institute) and R software (version 3.4.0) and were performed with a significance level at $p < 0.05$. A p -value < 0.1 was considered a statistical trend.

4. Results

Of the 518 women with analgesic intake data in each trimester, 245 women (47.3%) took an analgesic at least once during pregnancy, of which 210 (85.7%) took Paracetamol (Fig. 1). Table 1 presents the study participants characteristics separately for women taking no analgesics and those taking Paracetamol at least once during pregnancy. As expected, there was a tendency towards a higher percentage of women with preexisting medical conditions ($p = 0.080$) and allergies ($p = 0.062$) among women taking Paracetamol. Also, the percentage of women with at least one child was higher among those women taking any Paracetamol ($p = 0.008$) as well as the percentage of women with pregnancy complications ($p = 0.043$). There were no other differences between the two groups regarding maternal and obstetrical characteristics.

4.1. Detailed Data on Paracetamol Intake During Pregnancy

Among women taking analgesics, the percentage of women using Paracetamol increases throughout pregnancy, while the percentage of

women taking ibuprofen, the second most relevant analgesic drug, decreases (Supplemental Fig. 2). Of interest, between 9% (in the first and third trimester) and 14% (in the second trimester) of the women taking Paracetamol reported to have also taken at least one other analgesic, either as a combined product (i.e. with Hyoscine butylbromide or acetylsalicylic acid) or by taking an additional drug. Table 2 presents data regarding intake doses and duration for those women taking Paracetamol in the respective trimester if available. Overall, the majority of women took ≤ 500 mg Paracetamol/day and fell into the category of onetime users. Of note, the percentage of women in these groups decreased throughout the course of pregnancy. Concomitantly, an increasing percentage of women took between > 500 and 1500 mg Paracetamol (18.0% in the first and 29.1% in the third trimester). Also, the percentage of women taking Paracetamol over at least three continuous days increased from 18.7 to 33.3% in the third trimester. In accordance with these observations, both, the absolute Paracetamol dose and number of days per trimester with Paracetamol intake indicated that the majority of women took Paracetamol in low doses and at few days per trimester, although also higher intake doses and longer intake durations were observed for few women (Table 2).

4.2. Associations with Cord Blood Stem Cell Populations

Of 483 pregnancies with either no analgesic intake or Paracetamol intake, HSC cord blood data was available for 146 women (Fig. 1). These women did not differ with regard to Paracetamol intake ($p = 0.21$). In general women donating cord blood at the UKE had a slightly lower pre-pregnancy body-mass-index and delivered more boys compared to non-donors giving birth in another clinic (Supplemental Table 1).

The upper part of Table 3 presents the results of the unadjusted analyses of the association between the binary exposure (Paracetamol intake vs. no analgesic intake) and HSCs in cord blood. For the frequency of $CD34^+$ in $CD45^{int}$ cells, a statistical trend for an inverse association was observed: Women taking any Paracetamol had the tendency to have a lower percentage of $CD45^{int}CD34^+$ cells ($p = 0.075$). As a weaker association was observed for total HSCs ($p = 0.14$), this population was not considered further. In a next step, the predefined hypotheses for effects over the course of pregnancy were assessed: The least angle regression revealed third trimester Paracetamol intake (Paracetamol intake vs. no analgesic intake) as those variable being most correlated with the frequency of $CD45^{int}CD34^+$ cells. Looking at the elbow plot

Table 1
Maternal and obstetric characteristics presented for women taking no and any Paracetamol during pregnancy ($n = 483$).

	<i>n</i>	No Paracetamol intake ($n = 273$)	Any Paracetamol intake ($n = 210$)	<i>p</i> for difference ^a
Maternal characteristics				
Maternal age (years) ^b	483	32.35 (3.49)	33.41 (3.81)	0.47
Maternal first trimester BMI (kg/m ²)	483	24.17 (3.89)	25.04 (4.61)	0.15
Maternal education, <i>n</i> (%)	469			0.11
Main or middle school		52 (19.6%)	48 (23.7%)	
High school graduation		74 (27.8%)	69 (34.0%)	
University degree		140 (52.6%)	86 (42.4%)	
Pre-existing medical conditions, <i>n</i> (%)	468	56 (21.1%)	57 (28.1%)	0.080
Asthma, <i>n</i> (%)	479	3 (11.4%)	21 (10.1%)	0.88
Allergies, <i>n</i> (%)	478	128 (47.2%)	112 (54.1%)	0.062
Obstetrical characteristics				
Parity ≥ 1 , <i>n</i> (%)	481	87 (32.1%)	92 (43.8%)	0.008
Pregnancy complication, <i>n</i> (%)	440	72 (27.3%)	64 (36.4%)	0.043
Gestational age at delivery (weeks)	446	40.26 (1.32)	39.86 (1.55)	0.85
Birth weight (g)	446	3520 (463)	3467 (487)	0.83
Fetal gender (girl), <i>n</i> (%)	450	133 (48.7%)	97 (53.3%)	0.26
Hematopoietic stem cell frequencies				
$CD45^{int}CD34^+$ (median (P25, P50))	146	0.75 (0.46, 1.10)	0.59 (0.35, 0.90)	0.0754
Total HSC (median (P25, P50))	146	0.81 (0.51, 1.26)	0.64 (0.43, 1.17)	0.1479

^a *t*-test for continuous variables and chi-square test for categorical variables.

^b Mean (SD) are presented unless indicated otherwise.

Table 2
Paracetamol intake doses and duration in each trimester of pregnancy^a.

	1st trimester	2nd trimester	3rd trimester
Paracetamol intake dose/day	<i>n</i> = 111	<i>n</i> = 114	<i>n</i> = 79
>0 to ≤500 mg	85 (76.6%)	78 (68.4%)	52 (65.8%)
>500–1500 mg	20 (18.0%)	33 (28.9%)	23 (29.1%)
>1500–4000 mg	6 (5.4%)	3 (2.6%)	4 (4.1%)
>4000 mg	–	–	–
Total intake dose (mg)			
Median (P25, P75)	500 (500, 1500)	1000 (500, 2000)	1000 (500, 2500)
Min, max	200, 28,000	250, 51,500	250, 22,000
Paracetamol intake duration^b	<i>n</i> = 107	<i>n</i> = 114	<i>n</i> = 81
One time	61 (57.0%)	60 (52.6%)	31 (38.3%)
Occasionally	22 (20.6%)	17 (14.9%)	19 (23.5%)
Weekly	4 (3.7%)	5 (4.4%)	4 (4.9%)
Daily	20 (18.7%)	32 (28.1%)	27 (33.3%)
Total days of intake			
Median (P25, P75)	1 (1, 2)	1 (1, 3)	2 (1, 4)
Min, max	1, 14	1, 25	1, 20

^a *n*-numbers refer to the number of participants for whom data on dose and/or duration was available in the respective trimester.

^b Definition of categories: occasionally (irregular acetaminophen intake), weekly (regular intake at least for two weeks per trimester), daily (at least for three continuous days per trimester).

(Supplemental Fig. 3), no sharp bend – i.e. “elbow” – can be observed, indicating a relevance also of the accumulated intake, first and second trimester intake (mentioned in order of relevance). Uni- and multivariable regression models were hence run for all variables and results are presented in the second part of Table 3: A statistically significant inverse association was observed for the association between third trimester Paracetamol intake and the frequency of CD45^{int}CD34⁺ cells ($p = 0.038$). After adjustment for maternal age, maternal BMI, parity, gestational age and birth weight, a trend remained ($p = 0.068$). For none of the other variables assessed (accumulated, first and second trimester intake) statistically significant associations were observed, neither in the unadjusted nor in the adjusted model.

Sensitivity analyses were applied to preclude confounding by severe infection as indicated by parallel antibiotics medication. Multivariate models were further adjusted for antibiotics medication and additionally, an analyses was run excluding those few women taking both paracetamol and antibiotics in one trimester. Results remained unchanged (data not shown).

To consider medication doses and duration, boxplots of CD45^{int}CD34⁺ cells are presented in groups of women taking ≤500 mg and >500 mg Paracetamol per day (Fig. 2a) and in groups of women taking Paracetamol only once, occasionally/weekly or daily (for ≥3 days continuously) (Fig. 2b) in the third trimester, latter being the variable with the strongest association. While a trend of a lower percentage of CD45^{int}CD34⁺ cells was observed for the group of an Paracetamol

intake of >500 mg/day as well as for the group with intake for ≥3 continuous days, the statistical test indicated no difference due to the low *n*-number per group ($p = 0.16$ and $p = 0.48$ for groups regarding intake doses and duration, respectively).

5. Discussion

This study presents detailed data of Paracetamol intake during pregnancy. We observed that Paracetamol was by far the main analgesic taken by the pregnant women, underlining the relevance to investigate possible side effects. Indeed, we identified the third trimester as a critical period where Paracetamol intake was prospectively associated with a lower frequency of HSCs in cord blood.

In Germany, Paracetamol is an OTC drug, available only in licensed pharmacies. This is important to consider when comparing our observational data with i.e. US-data, where Paracetamol can be freely purchased in drug stores. Moreover, the current study sample is characterized by a high socioeconomic status, and may thereby not represent the general population. Comparing the general maternal characteristics of women who took Paracetamol with the non-analgesic group, a background in pre-existing medical conditions, such as allergies seem to favor the intake of analgesics. Furthermore, women expecting their 2nd child had an increased risk of taking Paracetamol, which might be due to a different lifestyle setting (infections of firstborn and elevated stress level). Although almost half of the pregnant women in our cohort took an analgesic, overall doses and days of intake were low, and no participant exceeded the recommended maximum daily intake dose of 4000 mg. Those few women pursuing Paracetamol intake in higher doses over longer durations experienced pregnancy complications, such as infections, and Paracetamol intake was clinically indicated. The need of higher dosed analgesic treatment increased with ongoing pregnancy and women took Paracetamol more frequently, while onetime usage decreased concomitantly. This may go along with a more continuous pain history with ongoing pregnancy, such as an increased weight burden, resulting in e.g. back ache. Of note, our data does not allow to distinguish between Paracetamol medication due to infections or pain as indication for analgesic treatment was not assessed. We are however aware of the possible association between maternal infections and offspring asthma development and therefore precluded a possible confounding effect of antibiotics medication (Zhu et al., 2016).

Several studies implied a long term effect for the children faced with Paracetamol in utero. Consequences such as an increased asthma risk, or Attention Deficit Hyperactivity Disorder were discussed (Liew et al., 2014; Eyers et al., 2011). Although strong interdependencies were shown, the actual literature lacks a prominent immunological explanation for the effects observed. We therefore provided a potential missing link by analyzing cord blood by flow cytometry and searched for variance in HSC populations. As Paracetamol intake varied greatly with respect to intake dose and duration, the main analyses in this study focused on binary and categorical analyses. However, by comparing

Table 3
Univariate and multivariate linear regression models on the association between Paracetamol intake and hematopoietic stem cell populations in cord blood ($n = 146$).

	β	95% CI	R ²	<i>p</i>
Univariate analyses for whole pregnancy (exposure: any Paracetamol intake yes/no):				
HSC total	−0.18589	−0.43350, 0.06172	0.012	0.14
CD45 ^{int} CD34 ⁺	−0.21478	−0.45161, 0.02205	0.021	0.075
Detailed analyses for the outcome CD45^{int}CD34⁺				
3rd trimester intake – unadjusted	−0.32382	−0.62870, −0.01894	0.024	0.038
3rd trimester intake – adjusted	−0.28553	−0.59200, 0.02093	0.086	0.068
accumulated intake – unadjusted	−0.08492	−0.22152, 0.05167	0.010	0.22
accumulated intake – adjusted	−0.07239	−0.20915, 0.06437	0.081	0.30
1st trimester intake – unadjusted	−0.02315	−0.32143, 0.27513	0.0001	0.88
1st trimester intake – adjusted	0.01595	−0.27702, 0.30893	0.068	0.91
2nd trimester intake – unadjusted	−0.15880	−0.43633, 0.11874	0.007	0.26
2nd trimester intake – adjusted	−0.16157	−0.43585, 0.11272	0.075	0.25

Models adjusted for maternal age, maternal BMI, parity, gestational age, birthweight.

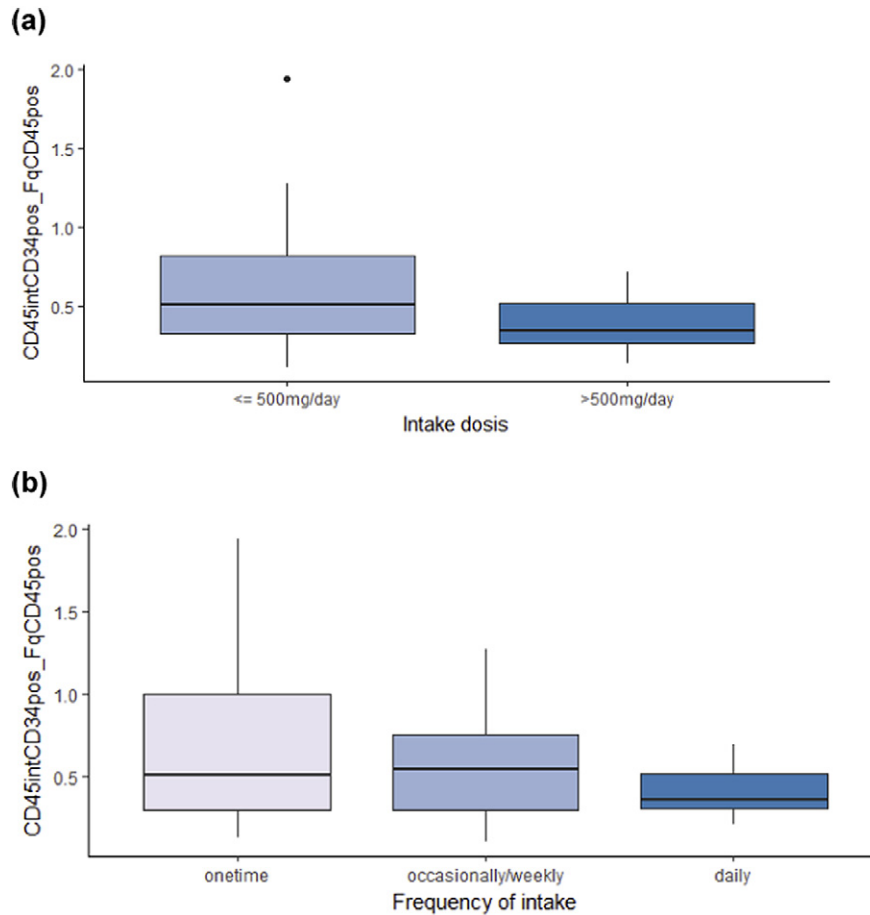


Fig. 2. Boxplots presenting the frequency of CD45^{int}CD34⁺ in CD45^{+int} in groups of women (a) taking ≤500 mg ($n = 17$) and >500 mg Paracetamol per day ($n = 7$) and (b) taking Paracetamol only once ($n = 8$), occasionally/weekly ($n = 8$) or on ≥3 consecutive days in the third trimester ($n = 9$) (definition of categories: occasionally (irregular acetaminophen intake), weekly (regular intake at least for two weeks per trimester), daily (at least for three continuous days per trimester)). Test for difference (Mann-Whitney-*U* Test and Kruskal-Wallis-Test) indicated no difference between the groups presented ($p = 0.16$ for groups regarding intake doses, and $p = 0.48$ for groups regarding intake duration).

the effect of no to any Paracetamol intake during pregnancy, we observed that the frequency of HSCs in cord blood was diminished when Paracetamol was taken. A more in depth analysis of our longitudinally collected data identified the 3rd trimester as the most critical trimester for Paracetamol related alterations of the HSC population. Looking at the overall explained variance of our models, even after consideration of relevant covariables, the total explained variation in cord blood stem cell populations ranged between 7 and 10%. This indicates that there are other factors impacting on the frequencies of these immune cells, which are independent of Paracetamol intake. The present results hence need to be interpreted carefully and should be seen as a moiety in the immunological setting. During immune adaptation to pregnancy, the maternal immune system is biased towards a Th2 cytokine response. This is also reflected in the cord blood and an additional Th2 bias upon Paracetamol use may be camouflaged by the already high Th2 levels. Moreover, it is controversial if cord blood IgE levels are predictable for atopy in the infant in the absence of atopic disease of the mother. (Pesonen et al., 2009) In future studies, we aim to determine a potential remaining Th2 skew and an allergen-specific IgE increase upon prenatal Paracetamol use in the children, which may account for an increased risk for asthma. Moreover, the reduced number of HSC we observed in cord blood upon Paracetamol use during pregnancy could be due to a reduced proliferation or increased death of HSC, which may subsequently affect myelo- and lymphopoiesis and hence, immunity in the child.

However, when third trimester intake doses and duration was additionally taken into account, our data indicated some effect of higher daily intake doses as well as a more continuous regular intake (Fig. 2).

However, small numbers per group precluded us from detecting significant dose-dependent effects. Hence, larger group sizes would be needed, which are difficult to obtain in observational studies due to ethical considerations. Nevertheless, these findings imply that even within the range of the maximum daily dosage the application of Paracetamol may alter immune relevant cell frequencies. The finding of a special relevance of third trimester Paracetamol intake bears tremendous clinical implications, as Paracetamol is considered one of the few analgesics safe to use in the last trimester of pregnancy. Other findings supported that the intake in the third trimester seems to be a crucial hallmark in NSAID therapy (Tanaka et al., 2016). Concordant to our findings a UK based study found that 3rd trimester intake of Paracetamol bears the highest risk of asthma development (Shaheen et al., 2005). In a study by Shaheen et al., the application of Paracetamol during the third trimester was correlated with wheezing (Shaheen et al., 2002). Importantly, considering also our observation of increased intake dosages taken during the 3rd trimester, effects on the HSCs might become even more prominent.

The underlying mechanism for a particular relevance in the third trimester remains elusive. It is tempting to speculate on a time dependent relation, or an easier Paracetamol passage through the placenta due to change in the vascularization. Structural alterations of capillaries to sinusoids, to meet the needs of the fetus, might additionally favor the transmission of Paracetamol (Milovanov et al., 2012). Paracetamol has been shown to be flow-limited in its transmission through the placenta (Nitsche et al., 2017). Whether hydrodynamics or epithelial factors in an aging placenta support an altered Paracetamol transmission has yet to be elucidated. Nevertheless, Paracetamol is known to pass the placenta freely and can easily reach sites of HSC in the fetus, e.g. the liver

(Rollins et al., 1979; Tiegs et al., 2014). Application of Paracetamol in pregnant mice has been shown to decrease HSC frequencies in the fetal liver (Karimi et al., 2015; Thiele et al., 2015), which may be due to apoptosis or reduced proliferation. Thus, it has been proposed that fetal HSC sites may be particularly susceptible to Paracetamol-induced hepatotoxicity, subsequently leading to a decline of HSCs. Such altered HSC pool may have consequences for the differentiation of HSCs in the different cell lineages, hereby affecting immunity of the offspring.

6. Outlook

The present results add valuable detailed data on Paracetamol intake during pregnancy and indicate a particular relevance of third trimester Paracetamol intake, which was prospectively associated with a reduced frequency of HSCs. Future studies are needed to disentangle potential trimester-specific effects and elucidate the impact of maternal medication onto the offspring's health with a special focus on immune development. We finally would like to stress that we acknowledge the importance of Paracetamol medication during pregnancy to prevent more severe consequences caused by fever and infections. There might however be circumstances, where Paracetamol is rather taken out of habit and because it is a cheap and generally available medication. From our perspective it is important to tackle these cases through education on possible adverse side-effects of Paracetamol during pregnancy.

Funding

This study was supported by Deutsche Forschungsgemeinschaft via KFO 296 (Grant: TI169/11-1 and DI-2103/2-1). The funding agency was not involved in any further scientific decision.

Conflicts of Interest

The authors declare no conflict of interest.

Author Contributions

LB and JG analyzed and interpreted the data, searched the literature, and wrote the manuscript; LB drafted the graphical abstract; JG conducted the figures; CW analyzed the data and conducted the figures; MP recruited the participants and collected the data; CG collected the FACS data and prepared the figure; HB supervised the data analysis; KH and PA conceived the study; AD and GT supervised the project; all authors critically revised the manuscript and approved the final version.

Acknowledgement

We are indebted to all participants of the PRINCE study for their collaboration. We further would like to thank Gudula Hansen and Dr. Franziska Rüber for their effort in recruiting the participants and collecting the data, Lesley-Ann Straub and Martin Piontek for data entry, Agnes Wieczorek, Nora Kersten, Romy Hackbusch and Christopher Urbshat for FACS work and Detlev Riller for valuable help with the graphical abstract. We thankfully acknowledge the FACS Core Facility at the Research Campus for their technical assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2017.10.023>.

References

- Brune, K., Renner, B., Tiegs, G., 2015. Acetaminophen/paracetamol: a history of errors, failures and false decisions. *Eur. J. Pain Lond. Engl.* 19, 953–965.
- Cheelo, M., Lodge, C.J., Dharmage, S.C., Simpson, J.A., Matheson, M., Heinrich, J., et al., 2015. Paracetamol exposure in pregnancy and early childhood and development of childhood asthma: a systematic review and meta-analysis. *Arch. Dis. Child.* 100, 81–89.
- Damkier, P., Scialli, A.R., Lusskin, S.I., 2017. Acetaminophen in pregnancy and adverse childhood neurodevelopment. *JAMA Pediatr.* 171, 396.
- Diemert, A., Hartwig, I., Pagenkemper, M., Mehnert, R., Hansen, G., Tolosa, E., et al., 2016. Fetal thymus size in human pregnancies reveals inverse association with regulatory T cell frequencies in cord blood. *J. Reprod. Immunol.* 113, 76–82.
- Diemert, A., Goletzke, J., Barkmann, C., Jung, R., Hecher, K., Arck, P., 2017. Maternal progesterone levels are modulated by maternal BMI and predict birth weight sex-specifically in human pregnancies. *J. Reprod. Immunol.* 121, 49–55.
- Eyers, S., Weatherall, M., Jefferies, S., Beasley, R., 2011. Paracetamol in pregnancy and the risk of wheezing in offspring: a systematic review and meta-analysis. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* 41, 482–489.
- Karimi, K., Keßler, T., Thiele, K., Ramisch, K., Erhardt, A., Huebener, P., et al., 2015. Prenatal acetaminophen induces liver toxicity in dams, reduces fetal liver stem cells, and increases airway inflammation in adult offspring. *J. Hepatol.* 62, 1085–1091.
- Liew, Z., Ritz, B., Rebordosa, C., Lee, P.-C., Olsen, J., 2014. Acetaminophen use during pregnancy, behavioral problems, and hyperkinetic disorders. *JAMA Pediatr.* 168, 313–320.
- Magnus, M.C., Karlstad, Ø., Håberg, S.E., Nafstad, P., Davey Smith, G., Nystad, W., 2016. Prenatal and infant paracetamol exposure and development of asthma: the Norwegian Mother and Child Cohort Study. *Int. J. Epidemiol.* 45, 512–522.
- Milovanov, A.P., Erofeeva, L.M., Aleksandrovich, N.V., Zolotukhina, I.A., 2012. Human placenta structure in II and III trimesters of physiological pregnancy. *Morfol. St. Petersburg Russ.* 142, 64–67.
- Nitsche, J.F., Patil, A.S., Langman, L.J., Penn, H.J., Derleth, D., Watson, W.J., et al., 2017. Transplacental passage of acetaminophen in term pregnancy. *Am. J. Perinatol.* 34, 541–543.
- Pesonen, M., Kallio, M.J.T., Siimes, M.A., Elg, P., Björkstén, F., Ranki, A., 2009. Cord serum immunoglobulin E as a risk factor for allergic symptoms and sensitization in children and young adults. *Pediatr. Allergy Immunol.* 20, 12–18.
- Rebordosa, C., Kogevinas, M., Sørensen, H.T., Olsen, J., 2008. Pre-natal exposure to paracetamol and risk of wheezing and asthma in children: a birth cohort study. *Int. J. Epidemiol.* 37, 583–590.
- Rollins, D.E., von Bahr, C., Glaumann, H., Moldéus, P., Rane, A., 1979. Acetaminophen: potentially toxic metabolite formed by human fetal and adult liver microsomes and isolated fetal liver cells. *Science* 205, 1414–1416.
- Saunders, N.R., Habgood, M.D., 2017. Acetaminophen in pregnancy and adverse childhood neurodevelopment. *JAMA Pediatr.* 171, 395.
- Shaheen, S.O., Newson, R.B., Sherriff, A., Henderson, A.J., Heron, J.E., Burney, P.G.J., et al., 2002. Paracetamol use in pregnancy and wheezing in early childhood. *Thorax* 57, 958–963.
- Shaheen, S.O., Newson, R.B., Henderson, A.J., Headley, J.E., Stratton, F.D., Jones, R.W., et al., 2005. Prenatal paracetamol exposure and risk of asthma and elevated immunoglobulin E in childhood. *Clin. Exp. Allergy J. Br. Soc. Allergy Clin. Immunol.* 35, 18–25.
- Shaheen, S.O., Newson, R.B., Ring, S.M., Rose-Zerilli, M.J., Holloway, J.W., Henderson, A.J., 2010. Prenatal and infant acetaminophen exposure, antioxidant gene polymorphisms, and childhood asthma. *J. Allergy Clin. Immunol.* 126 (1141–8.e7).
- Smith, A.D.A.C., Heron, J., Mishra, G., Gilthorpe, M.S., Ben-Shlomo, Y., Tilling, K., 2015. Model selection of the effect of binary exposures over the life course. *Epidemiol. Camb. Mass.* 26, 719–726.
- Stergiakouli, E., Thapar, A., Davey Smith, G., 2016. Association of acetaminophen use during pregnancy with behavioral problems in childhood: evidence against confounding. *JAMA Pediatr.* 170, 964–970.
- Tanaka, S., Hori, S., Satoh, H., Sawada, Y., 2016. Prediction of fetal ductus arteriosus constriction by systemic and local dermatological formulations of NSAIDs based on PK/PD analysis. *Int. J. Clin. Pharmacol. Ther.* 54, 782–794.
- Thiele, K., Solano, M.E., Huber, S., Flavell, R.A., Kessler, T., Barikbin, R., et al., 2015. Prenatal acetaminophen affects maternal immune and endocrine adaptation to pregnancy, induces placental damage, and impairs fetal development in mice. *Am. J. Pathol.* <https://doi.org/10.1016/j.ajpath.2015.06.019>.
- Tiegs, G., Karimi, K., Brune, K., Arck, P., 2014. New problems arising from old drugs: second-generation effects of acetaminophen. *Expert. Rev. Clin. Pharmacol.* 7, 655–662.
- Zhu, T., Zhang, L., Qu, Y., Mu, D., 2016. Meta-analysis of antenatal infection and risk of asthma and eczema. *Medicine (Baltimore)* 95, e4671.