



# The association of potential stressors with hair steroids in parents with small children: The Ulm SPATZ health study

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## ABSTRACT

Previous studies have reported weak associations between questionnaire-based stress measurements and hair steroids. A stronger relationship may exist in highly stressed subpopulations or with stress brought up by novel or unpredictable situations. In the Ulm SPATZ Health Study, conducted in Ulm, Germany, baseline recruitment 04/2012 to 05/2013, we analyzed data of families enrolled shortly after childbirth. Mothers completed standardized questionnaires assessing sociodemographic, health- and family-life-related factors, and the Screening Scale of the Trier Inventory of Chronic Stress (TICS) at 6 months (T2) and 12 months postpartum (T3). Their current partners completed SSCS-TICS and an Effort-Reward Imbalance (ERI) Questionnaire obtained at 6 weeks postpartum (T1). Partners ( $n = 375$  at T1) and mothers ( $n = 654$  at T2 or T3) provided a 2 to 3-cm hair segment for hair analysis. Adjusted linear and cubic spline regressions were used to analyze (non-)linear relationships between potential stressors and hair cortisol (hairF) and hair cortisone (hairE) concentrations as well as the respective change scores between 12 months and 6 months. Lacking social recognition and high paternal work overload were significantly associated with paternal hairF in cubic spline models (test for overall association,  $\chi^2 = 8.24$ ,  $p = 0.041$ ,  $\chi^2 = 8.41$ ,  $p = 0.038$ ) but not in linear models. However, the association between ERI and hairF ( $\chi^2 = 7.54$ ,  $p = 0.059$ ) was marginally significant. Maternal education was related to maternal hairF and hairE at T2. No association was observed between maternal postpartum employment and hair steroids at T2 or T3. Conversely, we could show a relationship between some change scores of stress and hairE in mothers. Considering non-linearity and family-related stressors, there are few associations between questionnaire-based stress measurements and hairF or hairE. Novelty of stressors was not shown to be a relevant factor.

## 1. Introduction

Hair steroid concentrations have been implicated as potential indicators of hypothalamic-pituitary-adrenal (HPA) axis function. Yet, previous studies have reported only weak associations between questionnaire-based measurements of stress or related constructs and hair cortisol concentrations (hairF), details see in various systematic reviews (Bates et al., 2017; Stalder et al., 2017; Mustonen et al., 2018) and subsequent original reports (Janssens et al., 2017; Gerber et al., 2017; Herr et al., 2017; CORTisolNETwork (CORNET) Consortium et al., 2017; Bossé et al., 2018; Mayer et al., 2018; van der Meij et al., 2018). Evidence on the corresponding correlation with hair cortisone concentrations (hairE), the inactive metabolite of cortisol, is more scarce (Vanaelst et al., 2013; Scharlau et al., 2017; Chen et al., 2016).

Studies investigating hairF have reported statistically significant

associations only for specific subscales of the stress instrument used, e.g. (Herr et al., 2017). Furthermore, another recently published study pointed to a higher correlation of self-reported stress with hairF in a study population with a high workload (van der Meij et al., 2018). These studies suggest that hairF may be sensitive to specific types of stress or within defined at-risk sub-populations. A similar notion has already been expressed (Stalder et al., 2014, 2017) assuming that situations with more severe stress might exhaust the regulatory capacities of the HPA axis and manifest in long-term endocrine changes (McEwen, 1998). Inconsistent results across populations and stress domains may also imply the presence of non-linear associations between questionnaire-measured chronic stress and hair steroids (Wells et al., 2014). In a recent study (Mayer et al., 2018), a cubic growth curve model for hairF in response to internship stress showed the best model fit. Therefore, data analyses may require non-linear methods which can

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unmask varying strengths of associations by stress level. Another tentative explanation for a weak association between stress and HPA axis activation might be that contextual variables had not been sufficiently considered (Mayer et al., 2018). Apart from the nature of the stressor itself, HPA axis activation appears to reflect factors like novelty or unpredictability (Koolhaas et al., 2011; Diemer, 2017; Mayer et al., 2018) as HPA axis response seems to habituate to repeated exposure to the same stressor (Spencer and Deak, 2017). Finally, it has also been suggested that hairE might be a more robust marker of HPA activation than hairF (Stalder et al., 2013).

To add to current knowledge on the relationship between stress and hair steroids, we investigated associations between questionnaire-based measurements of stress and hairF and hairE among mothers and their partners within the first year after the birth of a child, a period characterized by new experiences and novel stressors, using several approaches. Hypothesizing stronger associations with some domains of chronic stress, we explored associations for various domains of chronic stress questionnaires with hairF and hairE and the respective change scores in mothers. Further, we investigated relationships between restarting maternal employment and both outcomes. To account for potential nonlinearity, cubic splines were used in models where stress exposure was assessed as a continuous variable. Finally, to obtain a more robust measure of HPA axis activity, data on both hairF and hairE were considered.

## 2. Material and methods

### 2.1. Study design and study population

Data were derived from the Ulm SPATZ Health Study, a birth cohort study recruited from the general population in Ulm, Germany. Families in whom the mother gave birth to a child at Ulm University Medical Center from 04/2012 to 05/2013 were asked to participate (participation rate 49%). Details of the baseline examination were described (Braig et al., 2015). The study was approved by the ethics board of Ulm University (No. 311/11). All participants gave written informed consent. The study was carried out in accordance with the Declaration of Helsinki.

### 2.2. Collection and laboratory measurement of maternal hair steroids

Hair was collected by participants themselves or with the aid of a family member from the posterior vertex region of the head at 6 weeks postpartum (T1, partners) and 6 (T2, mothers) and 12 months postpartum (T3, mothers). Participants were told to cut two strands of hair as close to the scalp as possible using scissors. Participants were already familiar with the procedure as it was previously performed by study personnel shortly after delivery (Braig et al., 2015). An illustrated leaflet was provided to ensure proper adherence to the sampling protocol. Further, high correlation of hairF between self-sampling and sampling performed by trained study personnel has been previously reported in another study (Ouellet-Morin et al., 2016). Hair samples were wrapped in aluminium foil, the scalp end was marked, and the samples were sent to the study center. The analyses were done using the most proximal 3 cm adjacent to the scalp in a laboratory experienced in hair sample analyses. Given an average hair growth rate of 1 cm per month, this corresponds with a period of three months prior to sampling. Following sensitivity analysis, in 27 men, hair sampled of 2 cm (26 men) or 2.5 cm (1 man) were included since these data did not change the results presented. Hair steroids were analyzed using the published liquid chromatography tandem mass spectrometry protocol (Gao et al., 2013).

### 2.3. Covariates

The following maternal sociodemographic characteristics and

health- and lifestyle-related information were assessed at baseline (T0) using a self-administered standardized questionnaire: Maternal age at delivery (years), maternal nationality (German, others), maternal education ( $\leq 9$  years, 10–11 years,  $> 11$  years of school education), maternal occupational position (leadership/professionals, intermediate position, skilled manuals/non-manuals, unskilled/semi-skilled, and self-employed), and parity (0 births,  $\geq 1$  birth). Maternal body weight was self-reported at 6 months and 12 months. Body mass index (BMI) was classified according to World Health Organization guidelines into normal ( $\leq 24.9$  kg/m<sup>2</sup>), overweight (25.0–29.9 kg/m<sup>2</sup>), and obese ( $\geq 30.0$  kg/m<sup>2</sup>). At T1, the mothers' current partners were asked to provide information on their age, nationality, education, and their occupational position as well as body weight and height. Coding of these variables was done in accordance with maternal data gathered at T0.

### 2.4. Questionnaire-based stress measurements during follow-up

Chronic stress was measured using the Screening Scale of the Trier Inventory of Chronic Stress (SSCS-TICS, 12 items) (Schulz and Schlotz, 1999), which assesses chronic concerns (4 items), lacking social recognition (2 items), work overload (3 items), excessive demands (2 items), and social stress (1 item). We calculated summative subscales reflecting specific domains of chronic stress. The three-month time frame of SSCS-TICS matches the time frame of steroid accumulation in each analyzed 3 cm hair segment. Items of the SSCS-TICS are scored from 0 (never) to 4 (very frequently). For total score, missing values were replaced by the mean of the remaining items if there was not more than one missing value per stress domain ( $n = 11/654$  in mothers at T2,  $3/654$  in mothers at T3,  $4/375$  in partners at T1). Cronbach's alpha was 0.76 in partners (T1) and mothers and at T2 and 0.77 in mothers at T3.

### 2.5. Further family-related stressors

At T2 and T3, mothers were asked how many hours per week they worked in paid jobs in each month prior to the assessment. Variables were created indicating if mothers had restarted work for more than 10 h/week (yes/no) at 5–6 months or 10–12 months postpartum (each yes/no). These mothers were contrasted to mothers who worked prior to delivery but had not restarted to work yet. Furthermore, mothers also indicated their partners' working hours/week in the 2 months before the partners' hair sampling and before partners filled in the effort-reward imbalance questionnaire (Siegrist et al., 2004) as one measurement of work-related stress. The effort-reward imbalance (ERI) score was calculated based on the ratio of the cumulative scale for effort to the cumulative scale for reward, with a correction for the different number of items in the two scales. The overcommitment component of the model was derived using 10 Likert-scaled items ranging from 1 (low) to 4 (high intrinsic effort) assessing need for approval, competitiveness, and inability to withdraw from work; Cronbach's  $\alpha$  was 0.71 (effort), 0.83 (reward) and 0.68 (overcommitment).

## 3. Statistical analysis

Valid data for both questionnaires and hairF in at least 7.5 mg of hair were obtained in 654 mothers at 6 or 12 months and 375 partners. Data were excluded if hairF or hairE were below the detection limit ( $< 0.09$  pg/mg) or  $> 90.0$  pg/mg, respectively (Gao et al., 2013), which occurred for hairF in  $n = 3$  in partners,  $n = 13$  in mothers at T2,  $n = 3$  in mothers at T3, for hairE in  $n = 8$  in mothers at T2. Further exclusion was done due to intake of glucocorticoid medication ( $n = 24$  mothers at T2,  $n = 20$  at T3,  $n = 1$  partner). HairF and hairE were log-transformed to approach a normal distribution. The baseline characteristics of the study population were described. Associations between stress domains and hairF and hairE were modeled using cubic restricted splines with knots at the 5<sup>th</sup>, 35<sup>th</sup>, 65<sup>th</sup>, 95<sup>th</sup> percentile and additionally using linear regression, both adjusted for maternal

**Table 1**  
Characteristics of the study population.

	Mothers (n = 654 mothers with hairF measurement at T2 or T3)		Partners (n = 375 with hairF measurement at T1)	
	n/N	%	n/N	%
<b>Age, years</b>				
≤ 25	22/654	3.4	5/375	1.3
26–35	464/654	71.0	192/375	51.2
≥ 36	168/654	25.7	178/375	47.5
<b>Nationality</b>				
German	566/652	86.8	342/373	91.7
Other	86/652	13.2	31/373	8.3
<b>Education</b>				
≤ 9 years education	33/647	5.1	45/373	12.1
10–11 years education	188/647	29.1	65/373	17.4
> 11 years education	426/647	65.8	263/373	70.5
<b>Occupational position</b>				
leadership/professionals	140/467	30.0	133/324	41.0
intermediate position	227/467	48.6	22/324	6.8
skilled manuals/non-manuals	80/467	17.1	107/324	33.0
unskilled/semi-skilled	10/467	2.1	58/324	17.9
self-employed	10/467	2.1	4/324	1.2
<b>Parity at delivery</b>				
0 births	381/653	58.4		
≥ 1 births	272/653	41.7		
<b>BMI</b>				
normal	370/589	62.8	180/370	48.7
overweight	156/589	26.5	153/370	41.4
obese	63/589	11.7	37/370	10.0
<b>hairF partner 6 weeks</b> (pg/mg, log.), Median (IQR)			375/375	1.28 (1.06, 1.64)
<b>hairF mother 6 months</b> (pg/mg, log.), Median (IQR)	612/654	1.56 (1.29, 1.96)		
<b>hairF mother 12 months</b> (pg/mg, log.), Median (IQR)	530/654	1.51 (1.21, 1.91)		
<b>hairE partner 6 weeks</b> (pg/mg, log.), Median (IQR)			375/375	2.17 (1.90, 2.44)
<b>hairE mother 6 months</b> (pg/mg, log.), Median (IQR)	615/654	2.00 (1.67, 2.28)		
<b>hairE mother 12 months</b> (pg/mg, log.), Median (IQR)	541/654	2.25 (1.90, 2.54)		

Abbreviations: T1: 6 weeks postpartum, T2: 6 months postpartum, T3: 12 months postpartum, BMI: body mass index, hairF: hair cortisol concentrations, hairE: hair cortisone concentration, IQR: Interquartile range.

education, season of hair sampling, and BMI. Thereby the Chi<sup>2</sup> statistic tests if the spline parameters are zero. In addition, asterisks are given indicating whether the association is non-linear. Further potential covariates (Braig et al., 2015) were not associated with both questionnaire-based instruments and hair steroid concentrations in separate bivariate models at  $p < 0.1$ . Sensitivity analyses restricted to primiparous mothers at baseline were performed for all models. Furthermore, change scores for hairF, hairE, and stress domains were calculated by subtracting the T2 from the respective T3 value. For modeling the associations between changes in hairF or hairE and the stress domains knots were located at the 5<sup>th</sup>, 20<sup>th</sup>, 40<sup>th</sup> and 80<sup>th</sup> percentile to improve the model fit in the extremely high values. All statistical analyses were performed with SAS® 9.4 (The SAS Institute, Cary, NC, USA).

#### 4. Results

Characteristics of mothers and partners with measurements of hairF

and hairE who were included in the final analysis dataset are shown in Table 1. Parents were mostly of German nationality and 71.0% of mothers but 51.2% of their partners were between 26 and 35 years of age. Maternal median hairF but not consistently hairE was higher than the respective concentrations in their partners (see Table 1).

Table 2a presents means and standard deviations of SSCS-TICS scores in the specific chronic stress domains. Additionally, Wald chi-squared test statistics and p-values indicative of statistical significance of mothers' and their partners' SSCS-TICS total as well as of the respective stress domains as predictors of hairF accounting for non-linear associations are given. A statistically significant non-linear association was observed for lacking social recognition and work overload among partners (see Fig. 1) and a similar association was seen by trend for the total TICS score. We observed a marginally significant association between effort-reward imbalance ( $\chi^2 = 7.54$ ,  $p = 0.059$ ) but not working hours of partners with hairF. In addition, apart from a marginally statistically significant association between the lacking social recognition and hairE ( $\chi^2 = 6.21$ ,  $p = 0.102$ ), no statistically significant relationship was observed between any other stress domain (see Table 2b), paternal working hours, effort-reward imbalance, or overcommitment and hairE.

Further, lower maternal education was statistically significant associated with lower maternal hairF (< 9 years of education vs. > 11 years:  $b_{adj} = 0.28$ ,  $p = 0.034$ ; 10–11 years vs. > 11 years:  $b_{adj} = 0.12$ ,  $p = 0.044$ ) and hairE ( $b_{adj} = 0.05$ ,  $p = 0.309$ ,  $b_{adj} = 0.46$ ,  $p < 0.001$ ) at T2 compared to the highest educational group. No relationship was seen between restart of maternal employment and hairF or hairE (see Tables 3a and 3b). Besides, the change score of mothers' chronic stress domains worries and work overload, assessed at T2 and T3, were significantly associated with changes in maternal hairE (see Supplement, Table 1). However, these changes may also be indicative of a hairE regression to the mean (see Supplement, Fig. 1) as mothers starting low on hairE at T2 had a higher increase of hairE than mothers starting with high hairE at T2.

A restriction to first time mothers, for whom novelty of stressors related to the changing family life should be more pronounced, did not change the null results in mothers (data not shown). Further, the associations of paternal chronic stress domains and hairF were no longer significant after restriction, so were the associations between change scores of chronic stress domains and hairE in mothers.

#### 5. Discussion

In this large birth cohort study recruited from the general population of mothers who gave birth within the only obstetrical hospital in the city of Ulm, we investigated associations of family-related and chronic stress with hair steroid concentrations among mothers and their partners under the novel circumstances of having given birth to a child and changing family life. Despite exploring several dimensions related to different types of stress, we observed only few associations in fathers and some associations in maternal change scores. Nevertheless, using cubic splines for non-linear association seemed more meaningful than linear regression for some domains of stress. Finally, restriction to families with firstborn children for whom we expected to have an increased impact of stress on HPA axis function due to novel stressors did not show stronger associations.

The present study has some limitations. First, study participation rate was only 49% of eligible mothers which may have implications on generalizability of our results compared to the general population. This moderate participation rate combined with a possible loss to follow-up of higher stressed mothers (in terms of less educated, younger, or multipara mothers) may have resulted in underestimated associations. Second, median hairF were lower compared to those presented by others (Staufenbiel et al., 2013) which may have potentially contributed to the few associations we observed in spite of our efforts to also investigate a specific population experiencing novel stressors.

**Table 2a**  
Chronic stress domains and hairF. Results of an adjusted cubic spline regression<sup>1</sup>.

	Partners			Mothers					
	6 weeks (n = 375) Mean, SD <sup>2</sup>	Association with partners' hairF Chi <sup>2</sup>	p	6 months (n = 612)		12 months (n = 530)			
				Mean, SD <sup>2</sup>	Association with maternal hairF Chi <sup>2</sup>	p	Mean, SD <sup>2</sup>	Association with maternal hairF Chi <sup>2</sup>	p
<b>Worries</b>	4.72, 3.12	3.75	0.290	3.55, 2.96	0.23	0.972	3.62, 3.12	1.41	0.751
<b>Lacking social recognition</b>	1.94, 1.84	<b>8.24</b>	<b>0.041**</b>	1.80, 1.69	0.70	0.872	2.00, 1.77	1.48	0.688
<b>Work overload</b>	4.76, 2.66	<b>8.41</b>	<b>0.038**</b>	4.50, 2.51	1.76	0.624	4.65, 2.59	1.43	0.698
<b>Excessive demands</b>	2.38, 1.36	2.17	0.538	1.92, 1.39	1.67	0.643	1.89, 1.40	1.16	0.762
<b>Social stress<sup>3</sup></b>	0.89, 0.87	–	–	0.83, 0.87	–	–	0.81, 0.87	–	–
<b>Overall SSCS-TICS</b>	50.12, 9.87	6.72	0.081**	47.65, 9.86	1.25	0.742	48.00, 10.37	0.33	0.954

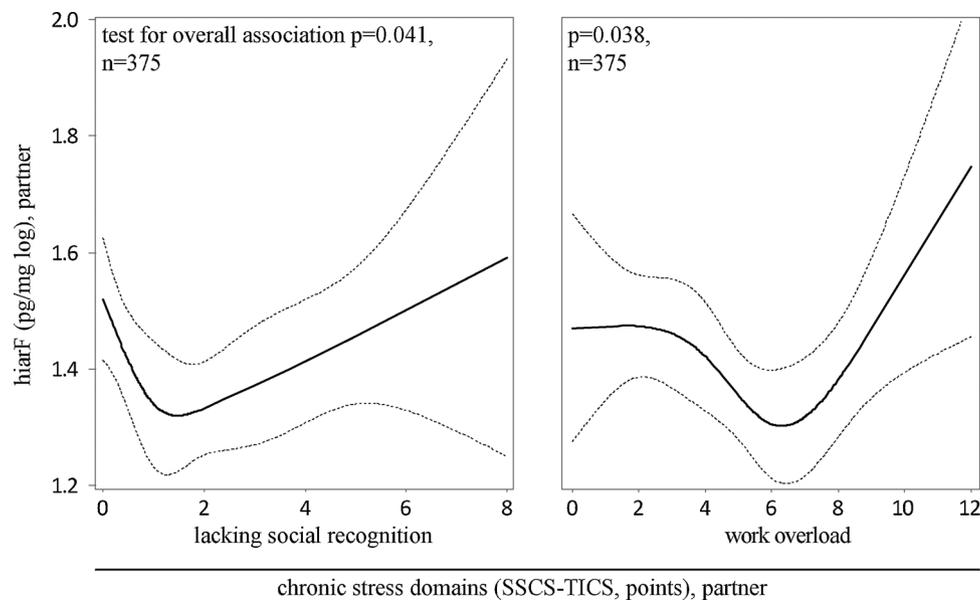
Abbreviations: hairF: hair cortisol concentrations, bold p values indicate a statistical significant association at  $p < 0.05$ .

<sup>1</sup> adjusted for education, seasonality, and BMI, absolute numbers may differ due to missing values.

<sup>2</sup> different numbers of items, means cannot be compared.

<sup>3</sup> as social stress only consists of one item cubic spline was not calculated.

\*\* significantly deviates from linear association at  $p < 0.05$ .



**Fig. 1.** Non-linear associations between chronic stress domains and hairF in partners.

Abbreviation: hairF: hair cortisol concentrations, SSCS-TICS: Screening Scale of the Trier Inventory of Chronic Stress, solid line: beta estimate, dashed lines: 95% Confidence Intervals

Notably, there is methodological variation in the laboratory analysis protocols leading to subsequent inter-laboratory variance in the absolute hairF or hairE (Stalder et al., 2017). Furthermore, intra-individual stability of hairF and hairE over time is lower than test-retest correlations previously reported in adult participants (Short et al., 2016; Stalder et al., 2012b) with correlation coefficients ranging from 0.13 to 0.37 (hairF) and 0.11 to 0.33 (hairE) when also considering maternal hair samples collected directly after childbirth. The reasons for the relatively low intraindividual stability of steroid concentrations in the present study are unknown. One potential contributing factor could be seen in the fact that hair sampling was conducted by participants themselves rather than by study personnel. However, this is unlikely to be a major contributor since previous research suggests the principal validity of self-sampling of hair for steroid analyses (Ouellet-Morin et al., 2016) and bias due to sampling method should be non-differential. Additional research based on larger-scale cohort data comparing home samples to the regular research protocol of hairF or hairE also using non-linear models for statistical analyses is needed to more

definitively assess whether the sampling method may play a role. Another reason for the low correlation between the time points might be the physiology of steroid secretion during pregnancy and postpartum. Cortisol release during pregnancy is largely increased with potential implications for the correlation coefficients. Although we tried to control for further covariates specific for the first year after having given birth to a child such as breastfeeding, there could be further psychological or somatic stressors we were not able to consider in the analyses. Thirdly, SSCS-TICS may not be the perfect questionnaire to assess mothers' stressful experiences in the first year of motherhood as this questionnaire does not directly assess specific stressors during this time period, such as sleep deprivation, demands of caring for a newborn and the need to adapt to a new social role (George, 2005; Osman et al., 2014). However, we assume that SSCS-TICS should reflect mothers' insecurity and worries, the burden of caring for a newborn and social stress in terms of new responsibilities. Moreover, we cannot fully exclude that further difficulties like feeding problems or child health problems, which were not specifically covered by our questionnaire

**Table 2b**  
Chronic stress domains and hairE. Results of an adjusted cubic spline regression<sup>1</sup>.

	Partners		Mothers		12 months	
	6 weeks (n = 375)		6 months (n = 615)		(n = 541)	
	Association with partners' hairE		Association with maternal hairE		Association with maternal hairE	
	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p	Chi <sup>2</sup>	p
Worries	0.84	0.840	5.32	0.150	1.92	0.589
<b>Lacking social recognition</b>	6.21	0.102*	2.14	0.543	1.57	0.665
<b>Work overload</b>	2.04	0.564	3.08	0.380	2.80	0.423
<b>Excessive demands</b>	2.62	0.455	0.83	0.841	5.81	0.121
<b>Social stress<sup>2</sup></b>	–	–	–	–	–	–
<b>Overall SSCS-TICS</b>	0.74	0.863	2.62	0.453	2.07	0.559

Abbreviations: hairE: hair cortisone concentrations, bold p values indicate a statistical significant association at  $p < 0.05$ .

<sup>1</sup> adjusted for education seasonality, and BMI, absolute numbers may differ due to missing values.

<sup>2</sup> as social stress only consists of one item cubic spline was not calculated.

\* significantly deviates from linear association at  $p < 0.10$ .

may have affected parents' stress and steroid levels. Consequently, the question whether the inclusion of such parenthood-specific factors may result in a stronger association with hair steroids needs to be addressed in further research. We could not explicitly investigate the hypothesis that the activation of HPA axis declines or habituates to repeated exposure to the same stressors (Spencer and Deak, 2017) but we assumed that the associations between potential family-based stressors and hair steroid concentrations should be higher in mothers of firstborns than in mothers who had already had a child – an assumption which was not supported by our study results. Lastly, whether the association between the change scores in SSCS-TICS and hairE in mothers is meaningful needs confirmation in studies with multiple measurements of stress and hair steroids at different time points.

Despite these shortcomings, our study thoroughly accounts for several aspects potentially explaining the lack of psychoendocrine covariance with high statistical power due to large sample size. Furthermore, we could control for several potential confounders, overcoming limitations of previous studies. Finally, we carefully excluded outliers of hairF and hairE below detection limit ( $< 0.9$  pg/mg) and  $> 90$  pg/mg. This exclusion altered our results potentially due to the cubic spline models which are more sensitive to outliers than linear regression models. Including values  $> 90$  pg/mg limit attenuated the associations between lacking social support as well as effort-reward imbalance and hairF in partners to non-significance. Literature on hairF or hairE outliers is diverse, considering values  $\pm 3SD$  from the mean e.g. Abell et al., 2016; Bossé et al., 2018, 3 Interquartile ranges from the median (Chen et al., 2015), or concentrations above a specific threshold

**Table 3a**  
Adjusted associations between family-related conditions or family-related stressors and hairF<sup>1</sup>.

	n (%)	Association with maternal hairF 6 months		Association with maternal hairF 12 months	
		adj. b	p	adj. b	p
<b>Mother restarted employment right before T2</b>					
no	495 (92.7)	1 referent			
yes	39 (7.3)	0.04	0.739		
<b>Mother restarted employment right before T3</b>					
no	401 (83.7)	1 referent			
yes	78 (16.3)	–0.01		0.900	

Abbreviations: hairF: hair cortisol concentrations, b: regression coefficient, T2: 6 months postpartum, T3: 12 months postpartum.

<sup>1</sup> adjusted for education, seasonality, and BMI.

(e.g. Boesch et al., 2015; Dowlati et al., 2010; Gow et al., 2011). There is additional inconsistency concerning the handling of chronic conditions and medication in the study population. Further research could potentially benefit from a deeper understanding of extreme hairF or hairE. Our results support few statistically significant associations between TICS and hairF (see review Stalder et al., 2017). One study (Dettenborn et al., 2010), however, observed higher concentrations in some TICS dimensions and higher hairF in unemployed vs. employed persons. Still, no association was found between SSCS-TICS and hairF in a population of older adults (Feller et al., 2014) and in two smaller studies with 155 and 58 participants (Stalder et al., 2012a), though details on the respective domains were not reported. We showed increased hairF in men with high work load and ERI. Similarly, there is evidence of a positive association between ERI and hairF (Qi et al., 2014; Steinisch et al., 2014; van der Meij et al., 2018) although not all studies support this relationship (Gidlow et al., 2016; Janssens et al., 2017). Due to these inconsistent results and considering the sex differences we observed in our analyses, we conclude that stressors activating the HPA axis might be highly individual and strongly depend on the population analyzed. Which aspect of stress activates HPA axis may hardly be comparable across studies. Furthermore, hairE may correspond more closely to questionnaire-based stress reports than hairF (Scharlau et al., 2017). Non-linear associations between stressors and hairF were reported (Wells et al., 2014; Mayer et al., 2018; Ursache et al., 2017). We could show such an association between SSCS-TICS work overload, lacking social recognition, and hairF but not or only marginally statistically significant for other stress domains or the total TICS score. Especially after correction for multiple testing, these associations along with the association between lacking social recognition and hairE are no longer significant. However, the results support the notion of a stronger association between questionnaire-based measures of stress and hair steroid concentrations in the extreme parts of our population. Additionally, we observed an association between maternal education and hairF. There is preliminary evidence suggesting that parental socio-economic status (SES) is inversely related to child hairF (see review Gray et al., 2018), but results are inconsistent. Previous studies investigating potential associations between SES and hairF in adults have reported either null (Chen et al., 2013; Steptoe et al., 2017; Wosu et al., 2015) or inverse results (Serwinski et al., 2016). Another study (Ursache et al., 2017) observed lower parental education to be associated with higher parental (and child) hairF. In this paper, socio-economic disadvantages modified the relationship between the parents' hairF and stress experiences in a non-linear relationship.

## 6. Conclusion

Although we spent much effort on investigating several aspects potentially explaining lack of correspondence of questionnaire-based stress measures with hair steroids, covariance between questionnaire-based stress measurement and hair steroids is low and inconsistent. However, it is questionable if positive relationships between the

**Table 3b**  
Adjusted associations between family-related conditions or family-related stressors and hairE<sup>1</sup>.

	Association with maternal hairE 6 months		Association with maternal hairE 12 months	
	adj. b	p	adj. b	p
<b>Mother restarted employment right before T2</b>				
no	1 referent		1 referent	
yes	−0.06	0.546	0.02	0.810
<b>Mother restarted employment right before T3</b>				
no				
yes				

Abbreviations: hairE: hair cortisone concentrations, b: regression coefficient, T2: 6 months postpartum, T3: 12 months postpartum.

<sup>1</sup> adjusted for educations, seasonality, and BMI.

behavioral and biological measures are necessary to support validity of the hairF or hairE as marker of psychosocial distress. Potentially, they are measuring components of psychological distress that are uniquely different from self-reported symptoms. Future research should increasingly investigate highly stressed individuals and situations prone to novel, unexpected stress, the latter is to date rarely reflected in epidemiological studies.

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None

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#### Appendix A. Supplementary data

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