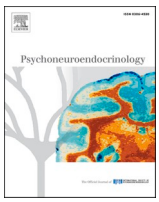
Psychoneuroendocrinology 154 (2023) 106309

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen



Hair cortisol, cortisone and DHEA concentrations and the composition of microbiota in toddlers

Maarit K. Koskinen [a,d,*], Anna Aatsinki [a,d], Susanna Kortesluoma [a,d], Paula Mustonen [a,d,f], Eveliina Munukka [b,d,e], Minna Lukkarinen [a,c,d], Laura Perasto [a,d], Anniina Keskitalo [a,d,e], Hasse Karlsson [a,d,g], Linnea Karlsson [a,c,d]

[a] FinnBrain Birth Cohort Study, Turku Brain and Mind Center, Department of Clinical Medicine, University of Turku, Turku, Finland
[b] Microbiology and Immunology, Institute of Biomedicine, University of Turku, Turku, Finland
[c] Department of Clinical Medicine, Paediatrics and Adolescent Medicine, University of Turku and Turku University Hospital, Turku, Finland
[d] Centre for Population Health Research, University of Turku and Turku University Hospital, Turku Finland
[e] Department of Clinical Microbiology, Turku University Hospital, Turku, Finland
[f] Department of Clinical Medicine, Child Psychiatry, University of Turku and Turku University Hospital, Turku, Finland
[g] Department of Clinical Medicine, Psychiatry, University of Turku and Turku University Hospital, Turku, Finland

ARTICLE INFO

Keywords:
Cortisol
Cortisone
DHEA
Hair sample
HPA axis
Microbiota

ABSTRACT

Animal research suggests that the gut microbiota and the HPA axis communicate in a bidirectional manner. However, human data, especially on early childhood, remain limited. In this exploratory design, we investigated the connections between long-term HPA axis functioning, measured as cortisol, cortisone or dehydroepiandrosterone concentrations and their ratios from hair segments of three centimeters, and gut microbiota profiles, (measured as diversity and bacterial composition by 16 S rRNA sequencing) in healthy 2.5-year-old toddlers (n = 135) recruited from the FinnBrain Birth Cohort Study. The alpha diversity of the microbiota was studied by linear regression. Beta diversity analyses with weighted UniFrac or Bray-Curtis distances were performed using PERMANOVA. The bacterial core genus level analyses were conducted using *DESeq*2 and ALDEx2. These analyses suggested that hair sample concentrations of separate hormones, cortisol/cortisone and cortisol/dehydroepiandrosterone ratios were associated with various gut bacterial genera such as the *Veillonella,* the [*Ruminococcus] torques* group *and [Eubacterium] hallii group,* although multiple testing correction attenuated the p-values. Alpha or beta diversity was not linked with either steroid concentrations or ratios. These findings in toddlers suggest that long-term HPA axis activity may be related to genera abundancies but not to ecosystem-level measures in gut microbiota. The influence of these observed interrelations on later child health and development warrants further research.

1. Introduction

As a sign of hypothalamic-pituitary-adrenal (HPA) axis activity, the end product cortisol is secreted from the adrenal cortex zona fasciculata layer of adrenal glands to the circulation. Cortisol secretion in humans has a diurnal pattern, with the peak measured during the morning and with a nadir at midnight. While salivary cortisol measures typically assess diurnal variability or stress reactivity, hair cortisol concentrations capture the long-term hormone secretion acitivity of the HPA axis (Stalder and Kirschbaum, 2012).

Cortisol exerts various effects on the human body, as it influences the immune system and metabolism (Russell and Lightman, 2019). Elevated levels of cortisol can be neurotoxic, while dehydroepiandrosterone (DHEA), also secreted from the adrenal glands, can offer neuroprotection (Kamin and Kertes, 2017). In fact, the cortisol/DHEA -ratio has been used to quantify the net effect of cortisol (Kamin and Kertes, 2017). Cortisol can also be converted to inactive cortisone by the enzyme 11 β-hydroxysteroid dehydrogenase type 2 (11β-HSD2)

Abbreviations: BMI SDS, body mass index standard deviation score; DHEA, dehydroepiandrosterone; FDR, false discovery rate; HCC, hair cortisol concentration; HPA, hypothalamic-pituitary-adrenal; SD, standard deviation.

* Correspondence to: Kiinamyllynkatu 10, 20520 Turku, Finland.
E-mail address: maarit.koskinen@utu.fi (M.K. Koskinen).

https://doi.org/10.1016/j.psyneuen.2023.106309
Received 1 December 2022; Received in revised form 24 May 2023; Accepted 24 May 2023
Available online 25 May 2023

(Tomlinson and Stewart, 2001). The cortisone and cortisol/cortisone ratio can be used as an estimate for cortisol metabolism by 11β-HSD2 (Dötsch et al., 2001). In addition, cortisol metabolites produced by gut microbiota act as 11β-HSD2 inhibitors (Morris and Brem, 2019).

Hair cortisol concentrations (HCC) are linked with a reduced nighttime sleep duration in infants (Flom et al., 2017) and attention-deficit-hyperactivity disorder (ADHD) in preschool boys (Mann et al., 2021). Hair DHEA associates with cognitive flexibility in kindergarten children (Pyle Hennessey et al., 2020) and the hair cortisol/DHEA ratio with depression in adolescent males (Kische et al., 2022).

In rodents, the gut microbiota composition is important for HPA axis development (Clarke et al., 2013; Sudo et al., 2004). The dynamics of the HPA response varies between mice harboring different microbiota compositions in their gut (Clarke et al., 2013; Sudo et al., 2004; Wu et al., 2021). Further, stress- or circadian disruption-induced changes in the HPA axis activity lead to subtle changes in gut microbiota, such as increased levels of the *Lachnospiraceae* family (El Aidy et al., 2020), the *Ruminococcus torques* species (Deaver et al., 2018), the genera *Clostridium* (Bailey et al., 2011) and *Alistipes* (Bangsgaard Bendtsen et al., 2012). Overall, these findings indicate that the gut microbiota composition and HPA axis activity are intertwined, although the exact mechanisms of the connections are unknown.

The microbiota composition and diversity change rapidly especially during infancy (Bäckhed et al., 2015). Alpha diversity, which describes the diversity and richness within the sample, increases with age (Fouhy et al., 2019; Roswall et al., 2021), and the prevalence and abundance of certain genera such as *Prevotella* and *Ruminococcus* increase over time in young children (Roswall et al., 2021). Studies on infants showdifferent connections between microbial components and cortisol measures, such as a negative correlation between alpha diversity describing variation within samples (calculated as the Shannon index) and saliva cortisol stress reactivity (Keskitalo et al., 2021). In one month old children there was a positive correlation between the genus *Prevotella* and saliva cortisol reactivity, and a negative correlation between the genera in the family *Lachnospiracea* and saliva cortisol reactivity (Rosin et al., 2021). In addition, hair cortisol concentrations show a positive correlation with *Ruminococci* in preadolescent children (Michels et al., 2019). Furthermore, beta diversity, describing the variation among samples and calculated as weighted UniFrac and Bray-Curtis dissimilarity indices, associate with the basal salivary cortisol at the age of three days (Jahnke et al., 2021). In newborns with low salivary cortisol, the abundance of *Enterobacteriaceae* was decreased, and the abundance of *Bifidobacterium* was increased (Jahnke et al., 2021).

In humans, the existing studies on early gut microbiota composition and cortisol concentrations have yielded varying results. There have been differences in analysis methods, covariate selection and inclusion, as well as how less prevalent genera were handled. To gain more knowledge on the connections between the long-term HPA axis activity and gut microbiota, we went on to study the long-term HPA axis activity in toddlers. Thus, the aim of this study was to investigate associations between fecal microbiota diversity and composition with long-term HPA axis activity in a subcohort of 2.5-year-old children from the FinnBrain Birth Cohort Study. The fecal microbiota diversity was estimated by using both alpha, calculated as Shannon and Chao1 indices, and beta diversities, calculated as weighted UniFrac and Bray-Curtis dissimilarity indices, and the composition was assessed using the abundances of the core genera. The long-term HPA axis activity was assessed by hair sample cortisol, cortisone and DHEA concentrations and relevant cortisol/cortisone and cortisol/DHEA ratios. Based on earlier studies using saliva cortisol, we hypothesized that cortisol, cortisone and DHEA concentrations would be related to microbiota composition, such as *Ruminococcus* and *Prevotella.*

2. Methods

2.1. Participants

The study children are from the FinnBrain Birth Cohort Study (Fig. 1), an ongoing pregnancy cohort study (n = 3808 families in baseline), where the main aim is to investigate the effects of early life stress on infant development (Karlsson et al., 2018). Out of the entire cohort, the recruitment for the 30-month study visit, where also hair and fecal sample collections were taking place for the current study, was primarily targeted to 1042 families who had data on prenatal measurements and longitudinal data on neuropsychological and parent-child interaction assessments (Holmberg et al., 2022) and were thus invited the follow-up visit. The total of 474 children participated the study visit with 324 giving the hair sample and 242 a fecal sample.

Hair samples were collected during the 30-month study visits, and fecal samples were collected at children's homes by the parents and brought into the research center according to a standardized protocol. The final study population of this particular report is a convenience sample of subjects with full overlapping data of hair samples and fecal samples (i.e. participation in the neuropsychological study visit, hair sample available and parents delivering the fecal sample and successful laboratory analysis of both biological samples).

The FinnBrain Birth Cohort Study has been approved by the Ethics Committee of the Hospital District of Southwest Finland. A written informed consent was provided by mothers for the study prior any measurements and tasks. Study recruitment from the general population was performed at gestational week (gwk) 12 from 12/2011–4/2015 in Southwest Finland and Åland. Children in the FinnBrain Study were regularly followed from birth at 3–36-month intervals (Karlsson et al., 2018). Children with a successfully analyzed gut microbiota profile from a fecal sample and an available hair sample at the age of 30 months were originally included in this study (n = 135, 67 boys, Table 1). Samples were collected as a part of the 30-month study visit that comprised of children participating in several tasks measuring child's temperament, cognition and evaluation of the parent-child interaction (Karlsson et al., 2018).

Data of covariates and possible confounders were collected through questionnaires and by linking register information to the study data. The gestational age and birth mode variables were retrieved from The Medical Birth Register by the Finnish Institute for Health and Welfare (www.thl.fi). Childrens' anthropometry data concerning the age of two years were based on the information from the yearly child welfare clinic visits, which parent had delivered to the FinnBrain pediatric study at the child age of 5 year. This visit included around 1000 children. Due to incomplete overlap among the participants from the 30-month and 5-year-old visits, there are randomly missing values in the BMI data of the current population of 30-month-olds. Data on pet ownership and on the child's daycare were retrieved from the parental questionnaire.

2.2. Hair samples

Hair samples and the related information were collected during the study visit at 30 months of age. The total number of hair samples at the 30-month visit was 294. Hair samples were collected as previously described (Mustonen et al., 2019) and proximal three-centimeter segments of hair were analyzed to assess the adrenal gland steroid accumulation of the previous three months. Hair cortisol, cortisone and DHEA were analyzed using mass spectrometry (Technical University of Dresden, Germany) with a protocol previously described in detail (Russell et al., 2015). To obtain a more complete picture of HPA axis functioning, we also combined cortisol with cortisone and DHEA in this study.

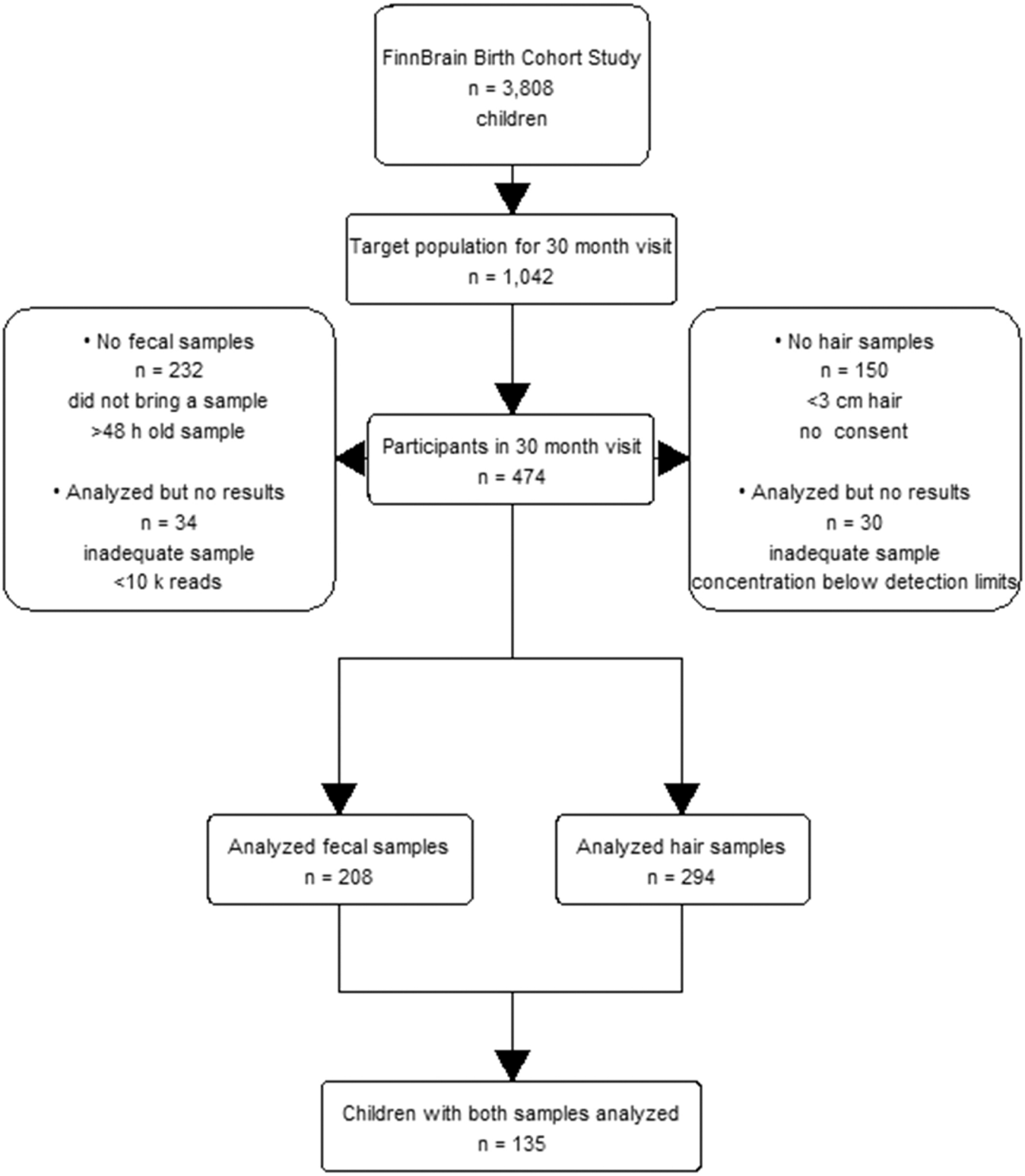


Fig. 1. The flow chart of the sample collection.

2.3. *The fecal samples*

Fecal samples for the analysis of gut microbiota studies were collected in sterile cups by parents at home, according to written and oral instructions as previously described (Keskitalo et al., 2021). Parents were informed to collect the sample close to the study visit and to store the samples at 4 °C prior to delivery. The total number of fecal samples from the 30-month visit was 208. Parents reported the sample collection times, and only samples delivered within 48 h of sampling were included for sequencing. Samples were stored at − 80 °C. The 16 S rRNA sequencing was performed using the Illumina Miseq platform (Illumina, San Diego, USA) using the V4 hypervariable region. The quality of the raw reads was checked with FastQC. The annotations for the Amplicon Sequence Variants (ASVs) were defined as part of the *DADA2* workflow

Table 1
Characteristics of the 2.5-year-old study children (n = 135).

Variable	Value
Child sex (male, n)	67
Gestational age in weeks, mean (SD)	40.0 (1.3)
Birth mode (n)	113
Vaginal	21
C-section	1
Missing data	
Mother's age at birth, mean (SD)	31 (4.4)
Mothers BMI before pregnancy (kg/m2), mean (SD)	24.5 (5.6)
Daycare (n)	47
Home	55
Daycare	3
Other	30
Missing data	
Pet ownership at 2 years of age (n)	62
No	40
Yes	33
Missing data	
BMI SDS, median (min-max)	0.04 (−2.91 to 2.46)
BMI category (n)	5
Significant underweight	3
Underweight	69
Normal	16
Overweight	6
Obese	36
Missing data	
Hair sample seasonal distribution (n)	35
Winter	37
Spring	32
Summer	31
Autumn	
Hair steroid concentrations* (pg/mg), median (min-max)	27.4 (1.6–5851)
Cortisol	39.4 (4.9–157)
Cortisone	7.4 (0.2–66.2)
DHEA	

BMI SDS, body mass index standard deviation score
SD, standard deviation
* Values included in the initial analyses (n = 132 for cortisol and DHEA; n = 133 for cortisone). Original median (min-max) cortisol, cortisone and DHEA concentrations were 28.4 (1.6–15156.9) pg/mg, 39.7 (4.9–214.7.0) pg/mg and 7.4 (0–169.2) pg/mg, respectively.

in combination with the SILVA database. *Phangorn* and *MSA* packages were used in the construction of the phylogenetic tree. Samples with less than 10 k total reads were omitted from this study resulting in 15347–381833 (median = 96269) reads.

2.4. Statistics

Statistical analyses were conducted with R statistical language and suitable packages including *microbiome*, *DESeq2* and *vegan*. The *Pheatmap* package (Kolde, 2019) was used in the construction of the heatmap (Fig. 3).

Hair cortisol, cortisone and DHEA values (below mean +3 SD, Table 1) as well as cortisol/cortisone and cortisone/DHEA ratios were, after log-transformation (Fig. 2), used as a measure for the long-term HPA axis activity and were used separately as an independent variable in models. Practices of the field regarding outlier treatment vary and the level of detail descriptions vary (Marceau et al., 2020). However, exclusion of values > 3 SD above the mean is based on unpublished expert consensus as it seems the correlations with hormone concentrations measured from other biomatrices such as saliva are diminished when hair hormone concentrations are extreme, which is assumed to result from, for instance, the activity of the 11β-HSD peripherally and other less well-known hair-related attributes. P-values of less than 0.05 were considered statistically significant. R scripts are found in Zenodo (doi: 10.5281/zenodo.7092636).

2.4.1. Confounders

After analyses without covariates, we adjusted the analyses for a priori selected covariates if they, based on the literature, potentially influenced both microbiota composition and analyzed hair steroid concentrations (Abell et al., 2016; Davenport et al., 2014; Gray et al., 2018; Jašarević et al., 2016; Le Chatelier et al., 2013). In children, less is known of the effect of seasonal variation on hair cortisol concentrations (Gray et al., 2018). In adults, however, different patterns of seasonal variation are observed potentially due to transpiration, temperature and perhaps seasonal variation in mood (Abell et al., 2016; Braig et al., 2015). Sunlight exposure may also play a role in seasonal variation in hair glucocorticoids (Wester et al., 2016). The seasonal variation observed in microbiota composition may be due to changes in diet (Davenport et al., 2014).

A directed acyclic graph (DAG, Supplementary Figure 1) was

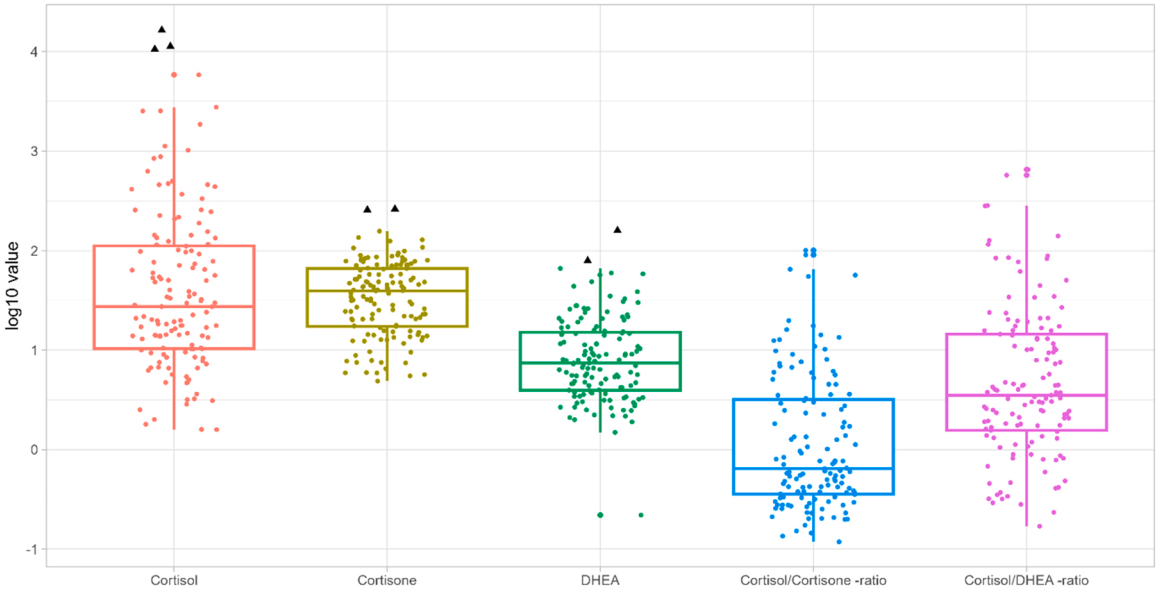


Fig. 2. Boxplots of log-transformed hair sample concentrations of cortisol, cortisone, DHEA (pg/mg) and cortisol/cortisone and cortisol/DHEA –ratios. Outliers not included in the analysis are shown with triangle (in addition, DHEA value 0 pg/mg is not shown).

constructed using *dagitty and ggdag*.

We chose a stepwise covariate inclusion as the sample size was limited regarding the BMI covariate information. The first analyses with covariates included the biological sex assigned at birth (henceforth sex) and season. Secondly, analyses were also performed with sex and two-categorical BMI. Lastly, analyses were performed with sex, season and categorical BMI. The sample seasons were categorized as winter (Dec–Feb), spring (March–May), summer (June–Aug) and autumn (Sep–Nov). The body mass index standard deviation score (BMISDS), which considers the sex and age of the child was categorized into two weight classes of normal/underweight vs. overweight/obese according to the Finnish growth references and definitions (Saari et al., 2011). The BMI class distribution differs from that of the FinnBrain Study as the prevalence of overweight and obesity was 39% in boys and 8% in girls in this study vs 29% in boys and 16% girls in the whole cohort (Hyppänen, 2020). The sex distribution and hair hormone concentrations are similar than in the whole FinnBrain Study cohort and in all of the hair samples from 30-month visit, respectively.

2.4.2. Alpha and beta diversity

Shannon and Chao1 indices were used for alpha diversity (*microbiome* package), and both indices were used as a dependent variable in

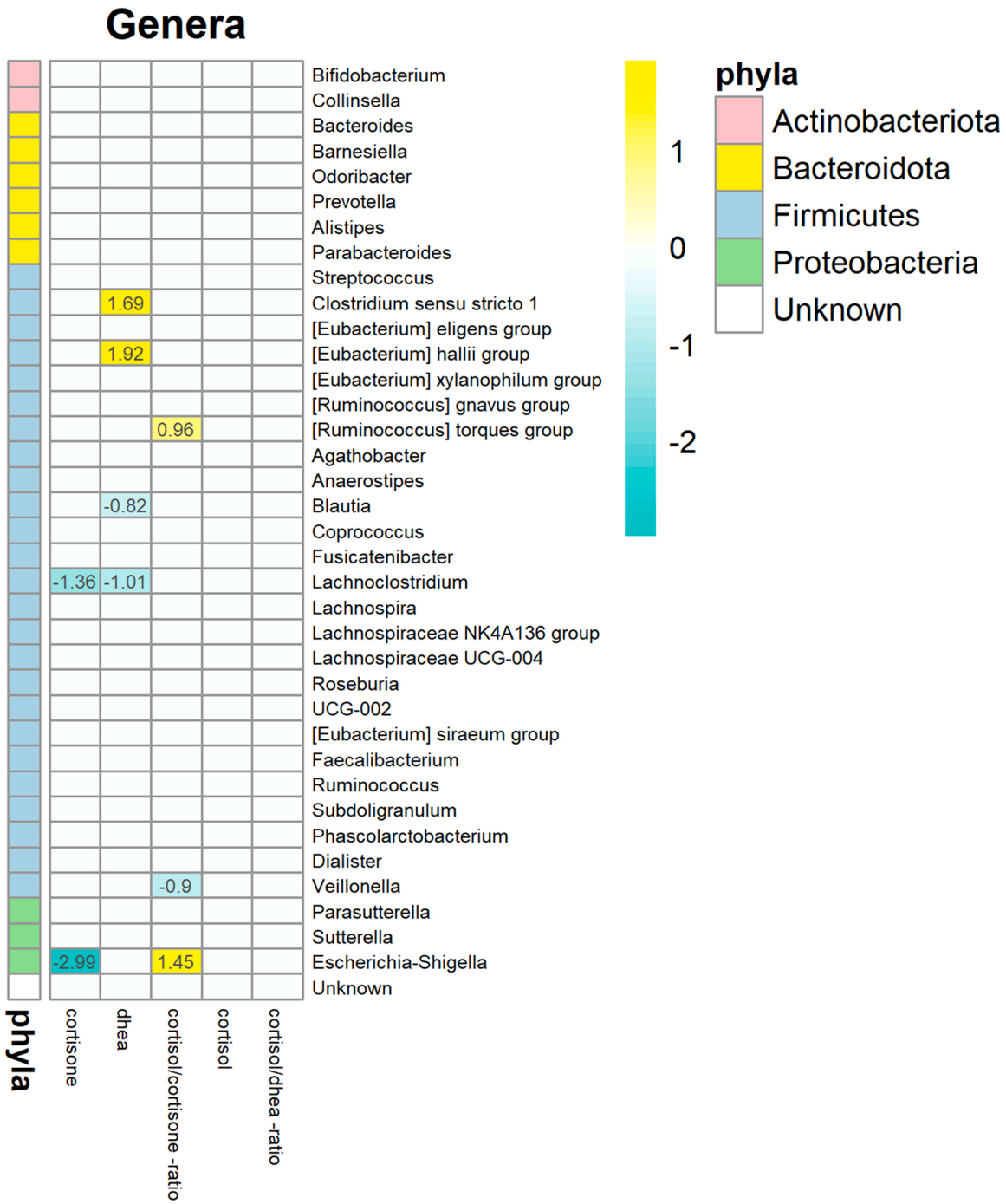


Fig. 3. In the final model (model 4), a total of 37 genera were involved in the composition analyses. The figure shows the $\log_2$fold values in the DESeq2 analysis for those genera (n = 9) that had a FDR < 0.25 in the final model when adjusting for season, sex and two categorical BMI. Genera that were excluded from part of the analyses (n = 2) or FDR > 0.25 are shown in white. FDR= false discovery rate, BMI=body mass index.

the linear regression models. Beta diversity calculations were based on Bray-Curtis dissimilarity and weighted UniFrac distances. Both indices take into account the relative abundance of taxa, and the UniFrac distance also uses the phylogenetic information. PERMANOVA analysis was performed using the adonis2 function in *vegan* package with 999 permutations. Marginal effects were calculated.

2.4.3. Differential abundance analyses

In the microbiota composition analyses with the differential abundances of core (prevalence>10%) genera (Fig. 3) *Deseq2* (Love et al., 2014) fitted a generalized linear model and reported the $\log_2$fold change per unit of change of the continuous variable. The 'poscounts' method uses positive counts for normalization. *DESeq2* uses a shrinkage estimation for dispersions ('local' fit was used). As this study was exploratory, the cutoff level of the Benjamini-Hochberg (BH) correction for multiple testing (false discovery rate, FDR) was 0.25 (meaning 25% are false positives) and was chosen to not overlook potential associations with any of the core genera. Analyses of the core genera were also repeated using ALDEx2, which is a more conservative method (Fernandes et al., 2014).

3. Results

Hair cortisol, cortisone and DHEA concentrations. In the samples included in the analyses, the median (range) hair cortisol, cortisone and DHEA concentrations were 27.4 (5850.4) pg/mg, 39.4 (152.1) pg/mg and 7.4 pg/mg (66.0), respectively (Table 1).

Alpha and beta diversity analyses. There was a high correlation between Shannon and Chao1 indexes (Spearman $\rho = 0.69$, $p < 0.0001$). No evidence was found for associations between child hair steroid concentrations, or their ratios and alpha or beta diversities of gut microbiota in unadjusted and adjusted analyses (Supplementary Tables 1 and 2).

Genus-level analyses. In the differential abundance analysis using DESeq2, the genera within phylae *Firmicutes*, *Actinobacteria* and *Bacteroidetes* showed both positive and negative associations with hair concentrations of cortisol and DHEA while hair cortisone associated negatively mainly with members of *Proteobacteria* and *Firmicutes* (Table 2). Overall, there was a positive association between the [*Ruminococcus] torques* group and cortisol and a negative association between the genus *Veillonella* and cortisol and positive associations with the genera *Prevotella* and [*Eucterium] hallii group* and DHEA in the first three models (Table 2). The negative association between *Escherichia-Shigella* and cortisone was still evident in the final model adjusting for sex, season and BMI class (model 4, Fig. 3), where the *Veillonella* and *[Ruminococcus] torques group* failed to show an association with cortisol in the final model. Instead, they showed a negative and positive association with the cortisol/cortisone ratio, respectively, and this ratio also showed a positive association with *Escherichia-Shigella* (Table 3). *Lachnoclostridium* showed a negative association with cortisone and a negative association with DHEA, and there was also a negative association between *Blautia* (phylum *Firmicutes*) and DHEA and a positive association between *Clostridium* sensu stricto *1* (phylum *Firmicutes*) and *Eubacterium hallii group* and DHEA in the final model.

Overall, in the analysis using *ALDEx2* (Supplementary Table 3), the analysis suggested associations mainly with members of *Firmicutes* even though the results were not significant after multiple correction. From the above-mentioned genera, the *[Ruminococcus] torques group* did not show a positive association with cortisol; instead, the analysis suggested that cortisone has a negative association with the *[Ruminococcus] torques group*, not *Escherichia-Shigella*, and a positive association with *Lachnospiraceae UCG-004*. *Veillonella* showed a preliminary association with the cortisol/cortisone ratio in the unadjusted model. The association with *Prevotella* and DHEA was seen in the second model where the greatest $\log_2$fold change was also seen using DESeq2. In the final model, ALDEx2 indicated associations between *Blautia* and the cortisol/cortisone ratio instead of DHEA (Supplementary Table 4 and 5). Lastly, the positive association with the *[Eubacterium] hallii group* and DHEA appeared similar using both methods (Supplementary Table 4), where the greatest estimates were seen in the last model.

The patterns of correlations seemed visually similar between long-term HPA axis functioning and centered log ratio (clr)-transformed abundances of selected genera based on previous analyses (*[Ruminococcus] torques group, Veillonella and [Eubacterium] hallii group*, Supplementary Figure 2).

4. Discussion

In this exploratory study, we hypothesized that the characteristics of fecal microbiota, i.e. microbial diversity and the abundances of certain genera would correlate with measures of long-term HPA axis activity. These measures included hair cortisol, cortisone and DHEA concentrations as well as cortisol/cortisone and cortisol/DHEA –ratios in 2.5-year-old children from the FinnBrain Birth Cohort Study. Our results suggest

Table 2
The genera with FDR< 0.25 in the DESeq2 differential expression analysis using the hair cortisol, cortisone and DHEA concentrations as independent variables.

	Cortisol			Cortisone			DHEA		
	genus	$\log_2$fold	FDR	genus	$\log_2$fold	FDR	genus	$\log_2$fold	FDR
Model 1	Ruminococcus torques group	0.83	0.031	Escherichia-Shigella	-3.7	< 0.0005	Bifidobacterium	-1.12	0.06
	Lachnospira	-0.94	0.031	Blautia	-0.75	0.21	(Eubacterium) hallii group	1.65	0.06
	Veillonella	-0.84	0.032	Lachnospira	-1.56	0.25	Prevotella	2.24	0.097
	Bifidobacterium	0.49	0.14						
Model 2	Veillonella	-0.86	0.031	Escherichia-Shigella	-2.85	0.005	Prevotella	4.2	< 0.0005
	Prevotella	1.10	0.17	Blautia	-0.82	0.065	Bifidobacterium	-0.92	0.24
	Lachnospira	-0.77	0.17	Lachnoclostrdium	-1.03	0.065	Eubacterium hallii group	1.3	0.24
	Ruminococcus torques group	0.61	0.19	Unknown	-1.39	0.065	Blautia	-0.62	0.24
	Alistipes	0.56	0.25						
Model 3	Ruminococcus torques group	0.93	0.19	Escherichia-Shigella	-3.56	0.0044	(Eubacterium) hallii group	1.85	0.11
	Veillonella	-0.81	0.19				Clostridium sensu stricto 1	1.60	0.11
							Prevotella	2.39	0.19
Model 4				Escherichia-Shigella	-2.99	0.032	Clostridium sensu stricto 1	1.69	0.10
				Lachnoclostridium	-1.36	0.037	(Eubacterium) hallii group	1.92	0.10
							Blautia	-0.82	0.11
							Lachnoclostridium	-1.01	0.11

Covariates: Model 1 without covariates; Model 2 with sex and season; Model 3 with sex and categorical BMI (normal/underweight vs overweight/obese); Model 4 with sex, season and categorical BMI (n = 132, n = 133, n = 132 in models 1 and 2; n = 98, n = 99, n = 97 in model 3; n = 98, n = 98, n = 97 in model 4 [cortisol, cortisone, DHEA respectively])
FDR= false discovery rate, BMI=body mass index

Table 3
The genera with FDR< 0.25 in the DESeq2 differential expression analysis using the cortisol/cortisone and cortisol/DHEA –ratios as independent variables.

	Cortisol/cortisone -ratio			Cortisol/DHEA -ratio		
	genus	Log_2fold	FDR	genus	Log_2fold	FDR
Model 1	Ruminococcus torques group	1.13	0.0061	Lachnospira	-1.14	0.010
	Veillonella	-1.05	0.0071	Veillonella	-0.91	0.010
	Lachnospira	-1.18	0.014	Ruminococcus torques group	0.85	0.022
	Bifidobacterium	0.64	0.051	Bifidobacterium	0.61	0.025
	Unknown	0.72	0.077			
	Alistipes	0.62	0.22			
Model 2	Veillonella	-1.14	0.0036	Veillonella	-0.93	0.012
	Prevotella	1.56	0.021	Lachnospira	-0.99	0.031
	Ruminococcus torques group	0.96	0.021	Bifidobacterium	0.47	0.15
	Lachnospira	-0.98	0.041	Prevotella	-1.07	0.15
	Escherichia-Shigella	1.12	0.041	Ruminococcus torques group	0.65	0.15
	Unknown	0.78	0.041	Alistipes	0.56	0.20
	Alistipes	0.77	0.052	Parabacteroides	0.75	0.21
	UCG-002	1.24	0.14			
	Bifidobacterium	0.46	0.17			
Model 3	Ruminococcus torques group	1.16	0.041	Ruminococcus torques group	0.99	0.15
				Veillonella	-0.83	0.20
Model 4	Veillonella	-0.90	0.095			
	Escherichia-Shigella	1.45	0.095			
	Ruminococcus torques group	0.96	0.095			

Covariates: Model 1 without covariates; Model 2 with sex and season; Model 3 with sex and categorical BMI (normal/underweight vs overweight/obese); Model 4 with sex, season and categorical BMI (n = 131, n = 129 in models 1 and 2; n = 98, n = 96 models 3 and 4 [cortisol/cortisone and cortisol/DHEA-ratios, respectively]). FDR= false discovery rate, BMI=body mass index

that long-term adrenal gland steroid concentrations and cortisol/DHEA and cortisol/cortisone ratios are associated with the abundance of certain gut microbial genera, while no associations with alpha or beta diversity were observed. Animal studies show that the manipulation or depletion of intestinal microbiota can alter the HPA axis development (Sudo et al., 2004; Wu et al., 2021). However, findings from human studies searching the possible links between markers of HPA axis functioning and microbiota have been inconsistent. Our results suggest that the preceding long-term HPA axis functioning is associated with fecal microbiota composition in 2.5-year-olds, and highlight the need for mechanistic studies on HPA axis functioning and gut microbiota.

Studies investigating the possible link between cortisol and the microbiome in children have used both saliva and hair samples. In humans, most of the circulating cortisol is bound to cortisol-binding globulin. Saliva samples measure free cortisol for the moment (Vining et al., 1983). Hair samples of cortisol, on the other hand, represent the free cortisol that has been secreted into hair from surrounding capillaries from months previously (Stalder and Kirschbaum, 2012). Our results were in part aligned with a study using hair samples for cortisol assessment as one *Ruminococcus* genus showed a positive association with hair cortisol in 8–16-year-old children and adolescents (Michels et al., 2019).

Taken partly from an overlapping cohort subpopulation, infant salivary cortisol acute stress reactivity did not relate to gut microbiota composition (Keskitalo et al., 2021). Another study suggests that the saliva cortisol reactivity shows positive correlation with the abundance of the genus *Prevotella,* but an opposite correlation with the genera in the family *Lachnospiracea* in one-month-old children (Rosin et al., 2021). These interrelations may be different over time as children develop. When newborns with either high or low saliva cortisol concentrations were compared, there were differences in the abundances of typical gut bacterial families such as *Enterobacteriaceae* and *Bifidobacterium* but these differences were not observed when analyzed again at two months of age (Jahnke et al., 2021)(. Long-term assessment methods may find different associations than studies using momentary approaches as these methods capture differential aspects of HPA axis functioning.

In DESeq2, hair cortisone showed the most consistent negative association with the genus *Escherichia-Shigella*, belonging to the *Enterobactericeae* family of facultative anaerobic gram-negative bacteria. The association was negative with cortisone, but positive with the cortisol/cortisone ratio. The most studied species within this genus, namely *Escherichia coli*, are linked with the stress response in mice, as cortisone concentrations in one-hour restraint stress were higher in enteropathogenic *E. coli* monoassociated mice compared to germfree mice. However, this was not seen when using mutant *E. coli* (Sudo et al., 2004). The increased inflammation due to bacterial internalization to the intestinal epithelial layer could affect the HPA axis activity (Sudo et al., 2004). While this could also apply to other genera, *Escherichia coli* is still a very typical member of gut microbiota. The factors determining the overall wellbeing of the gut, such as diet or existing inflammation could moderate the effect of gut microbiota on systemic health, as it has been reported that the translocation of *E. coli* strains multiplied after exposure to cytokines and nutrient depletion (Macutkiewicz et al., 2008).

In our study using DESeq2, the [*Ruminococcus] torques* group had consistently positive associations with cortisol and the cortisol/DHEA ratio, while *Veillonella* had a consistently negative association with cortisol and the cortisol/DHEA ratio despite the decrease in power in the models. In the final model, both of these associations with the *Veillonella* and [*Ruminococcus] torques* group were still evident with the cortisol/cortisone ratios. Interestingly, *Ruminococcus torques* has been previously linked with stress, as mice that were exposed to 4 weeks of circadian disruption had an increased abundance of this species (Deaver et al., 2018). *R. torques* has the ability to metabolize carbohydrate branches of mucin into less complex carbohydrates, and the abundance of this species was substantially higher in patients with inflammatory bowel disease compared to controls (Png et al., 2010). In a recent review, the genus *Veillonella* was increased within psychiatric disorders in all studies that it was reported (McGuinness et al., 2022). *Veillonella* annotated taxa also are linked with specific temperament traits in the 2.5-month-old FinnBrain Study children, such as fear reactivity in girls, where both positive and negative associations were found (Aatsinki et al., 2019). *Veillonellae* do not typically metabolize dietary carbohydrates but use lactate instead as their carbon source. After heavy exercise, their abundance was increased, and the family *Veillonellae* were the preferred microbe to metabolize lactate, producing propionate (Scheiman et al., 2019). Lactate has been studied in the context of panic attacks, but it has not been consistently linked with change in cortisol levels (Bandelow et al., 2017). On the other hand, propionate can be protective on the central nervous system (Hoyles et al., 2018), and lactate may also play an important role in metabolic signaling in the developing brain

(Monsorno et al., 2022).

The *[Eubacterium] hallii group* showed an association with DHEA using both differential abundance analysis methods. *Eubacterium hallii* is important in preventing lactate accumulation, and it can utilize lactate and acetate as well as glucose to form butyrate (Duncan et al., 2004). *Eubacterium hallii* is also capable of producing propionate (Engels et al., 2016). Like DHEA, which was connected with cognition in children (Pyle Hennessey et al., 2020), *E. hallii* also associates with cognition (Kong et al., 2021; Oluwagbemigun et al., 2022). The connection with DHEA has been speculated to be due to a decrease in the amygdalar- and hippocampal-based functions that could lead to more refined cognitive functions (Nguyen et al., 2017). In mice, short-chain fatty acids are implicated in cognition (Burokas et al., 2017; van de Wouw et al., 2018).

Overall, several taxa from the class *Clostridia* showed both positive and negative associations with hair sample concentrations of adrenal gland steroids. Interestingly, the members of class *Clostridia* take part in many physiological processes in the gut (Lopetuso et al., 2013). Especially the members of *Lachnospiraceae* and *Ruminococcaceae* are known to be important butyrate producers, and, in infants *Clostridium* sensu stricto was among the butyrate producing taxa (Vital et al., 2017). Butyrate has anti-inflammatory effects and it is also an energy source for colonic epithelial cells (Hamer et al., 2008). Butyrate has been negatively associated with cardiovascular diseases (Karlsson et al., 2012) and recently also with adult psychiatric disorders (Nikolova et al., 2021), as the abundance of certain butyrate-producing bacteria was decreased, and there was also an increase in butyrate depleting bacteria. High-dose administration of sodium butyrate causes an increase in cortisol levels (Gagliano et al., 2014).

By using hair samples, we were able to measure the long-term HPA axis functioning for approximately the three previous months. Combining cortisol with cortisone and DHEA in order to describe HPA axis functioning in more detail could be beneficial in future studies as some of the findings were not found by using only cortisol. We used different methods in the analyses searching for these associations. Microbial abundance results typically differ between methods, and some methods are more conservative (Nearing et al., 2022). Indeed, ALDEx2 with a low false-positive rate, but may additionally underestimate findings in the range of low p values (Calgaro et al., 2020).

In this study, we used hair sample concentrations consistently as independent variables in the models. Future studies should incorporate longitudinal samples to obtain a more complete picture of the temporal dynamics of the HPA axis, microbiota composition and possibly microbiota functional capacity or output. Moreover, the use of shot-gun sequencing instead of amplicon sequencing would help to disentangle the functional potential as well as give higher taxonomic resolution.

In conclusion, we observed several key associations with genera abundances although the long-term secretion of cortisol, cortisone or DHEA was not associated with fecal microbiota ecosystem-level characteristics. These findings corroborate findings from earlier studies (Jahnke et al., 2021; Michels et al., 2019; Rosin et al., 2021). They allude to the importance of inflammatory cytokines and microbial metabolites such as short chain fatty acids in the connection of gut-brain-axis homeostasis. These findings will hopefully pave the way for studies with higher granularity, and have the potential to make mechanistic hypotheses, by e.g., leveraging shot-gun sequencing and metabolomics. This knowledge can be used to understand the role of gut microbiota and HPA axis functioning in child development and health and develop interventions to support it. Future studies are warranted and aim to investigate the bidirectional nature of the gut-brain axis, whether alterations in HPA axis functioning impact gut microbiota communities longitudinally and vice versa and what the possible involved mechanisms are.

Funding

This study was funded by Academy of Finland #308176, #325292 and #308589, The Waterloo Foundation #2110-3601, The Signe and Ane Gyllenberg Foundation, Finnish State Grants for Clinical Research (ERVA) and The Finnish Medical Foundation. The funding sources had no role in the study design, data collection, analyses, interpretation of data, writing of the article, or in the decision to submit the manuscript for publication.

CRediT authorship contribution statement

Maarit Koskinen: writing – original draft, Writing – review & editing, Formal analysis. **Anna Aatsinki:** Supervision, Conceptualization, Writing – review & editing, Formal analysis. **Susanna Kortesluoma:** Investigation, Writing, – review & editing. **Paula Mustonen**: Investigation, Writing – review & editing. **Eveliina Munukka**: Investigation, Writing – review & editing. **Minna Lukkarinen:** Investigation, Writing, review & editing. **Laura Perasto:** Formal analysis. **Anniina Keskitalo**: Investigatio. **Hasse Karlsson:** Conceptualization, Project administration, Funding acquisition. **Linnea Karlsson:** Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of Competing Interest

EM is currently a Medical Advisor in Biocodex Nordics. Other authors report no conflicts of interest.

Acknowledgements

We thank the FinnBrain Birth Cohort Study personnel and all the families that have participated in the FinnBrain study. Tuomo-Artturi Autere is thanked for statistical support and data management and Teemu Kemppainen for data management. We also thank Robert M. Badeau, PhD, for providing English language proofreading services for this manuscript.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2023.106309.

References

Aatsinki, A.-K., Lahti, L., Uusitupa, H.-M., Munukka, E., Keskitalo, A., Nolvi, S., O'Mahony, S., Pietilä, S., Elo, L.L., Eerola, E., Karlsson, H., Karlsson, L., 2019. Gut microbiota composition is associated with temperament traits in infants. Brain Behav. Immun. 80, 849–858. https://doi.org/10.1016/j.bbi.2019.05.035.

Abell, J.G., Stalder, T., Ferrie, J.E., Shipley, M.J., Kirschbaum, C., Kivimäki, M., Kumari, M., 2016. Assessing cortisol from hair samples in a large observational cohort: the whitehall II study. Psychoneuroendocrinology 73, 148–156. https://doi.org/10.1016/j.psyneuen.2016.07.214.

Bäckhed, F., Roswall, J., Peng, Y., Feng, Q., Jia, H., Kovatcheva-Datchary, P., Li, Y., Xia, Y., Xie, H., Zhong, H., Khan, M.T., Zhang, J., Li, J., Xiao, L., Al-Aama, J., Zhang, D., Lee, Y.S., Kotowska, D., Colding, C., Tremaroli, V., Yin, Y., Bergman, S., Xu, X., Madsen, L., Kristiansen, K., Dahlgren, J., Wang, J., 2015. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell Host Microbe. 17, 690–703. https://doi.org/10.1016/j.chom.2015.04.004.

Bailey, M.T., Dowd, S.E., Galley, J.D., Hufnagle, A.R., Allen, R.G., Lyte, M., 2011. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav. Immun. 25, 397–407. https://doi.org/10.1016/j.bbi.2010.10.023.

Bandelow, B., Baldwin, D., Abelli, M., Bolea-Alamanac, B., Bourin, M., Chamberlain, S. R., Cinosi, E., Davies, S., Domschke, K., Fineberg, N., Grünblatt, E., Jarema, M., Kim, Y.-K., Maron, E., Masdrakis, V., Mikova, O., Nutt, D., Pallanti, S., Pini, S., Ströhle, A., Thibaut, F., Vaghi, M.M., Won, E., Wedekind, D., Wichniak, A., Woolley, J., Zwanzger, P., Riederer, P., 2017. Biological markers for anxiety disorders, OCD and PTSD: a consensus statement. part ii: neurochemistry, neurophysiology and neurocognition. World J. Biol. Psychiatry 18, 162–214. https://doi.org/10.1080/15622975.2016.1190867.

Bangsgaard Bendtsen, K.M., Krych, L., Sørensen, D.B., Pang, W., Nielsen, D.S., Josefsen, K., Hansen, L.H., Sørensen, S.J., Hansen, A.K., 2012. Gut microbiota composition is correlated to grid floor induced stress and behavior in the balb/c mouse. PLoS ONE 7, e46231. https://doi.org/10.1371/journal.pone.0046231.

Braig, S., Grabher, F., Ntomchukwu, C., Reister, F., Stalder, T., Kirschbaum, C., Genuneit, J., Rothenbacher, D., 2015. Determinants of maternal hair cortisol concentrations at delivery reflecting the last trimester of pregnancy. Psychoneuroendocrinology 52, 289–296. https://doi.org/10.1016/j.psyneuen.2014.12.006.

Burokas, A., Arboleya, S., Moloney, R.D., Peterson, V.L., Murphy, K., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2017. Targeting the microbiota-gut-brain axis: prebiotics have anxiolytic and antidepressant-like effects and reverse the impact of chronic stress in mice. Biol. Psychiatry 82, 472–487. https://doi.org/10.1016/j.biopsych.2016.12.031.

Calgaro, M., Romualdi, C., Waldron, L., Risso, D., Vitulo, N., 2020. Assessment of statistical methods from single cell, bulk RNA-seq, and metagenomics applied to microbiome data. Genome Biol. 21, 191. https://doi.org/10.1186/s13059-020-02104-1.

Clarke, G., Grenham, S., Scully, P., Fitzgerald, P., Moloney, R.D., Shanahan, F., Dinan, T. G., Cryan, J.F., 2013. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol. Psychiatry 18, 666–673. https://doi.org/10.1038/mp.2012.77.

Davenport, E.R., Mizrahi-Man, O., Michelini, K., Barreiro, L.B., Ober, C., Gilad, Y., 2014. Seasonal variation in human gut microbiome composition. PLoS ONE 9, e90731. https://doi.org/10.1371/journal.pone.0090731.

Deaver, J.A., Eum, S.Y., Toborek, M., 2018. Circadian disruption changes gut microbiome taxa and functional gene composition. Front. Microbiol. 9, 737. https://doi.org/10.3389/fmicb.2018.00737.

Dötsch, J., Dörr, H.G., Stalla, G.K., Sippell, W.G., 2001. Effect of glucocorticoid excess on the cortisol/cortisone ratio. Steroids 66, 817–820. https://doi.org/10.1016/S0039-128X(01)00117-9.

Duncan, S.H., Louis, P., Flint, H.J., 2004. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. Appl. Environ. Microbiol 70, 5810–5817. https://doi.org/10.1128/AEM.70.10.5810-5817.2004.

El Aidy, S., Bolsius, Y.G., Raven, F., Havekes, R., 2020. A brief period of sleep deprivation leads to subtle changes in mouse gut microbiota. J. Sleep. Res. 29. https://doi.org/10.1111/jsr.12920.

Engels, C., Ruscheweyh, H.-J., Beerenwinkel, N., Lacroix, C., Schwab, C., 2016. The common gut microbe eubacterium hallii also contributes to intestinal propionate formation. Front. Microbiol. 7. https://doi.org/10.3389/fmicb.2016.00713.

Fernandes, A.D., Reid, J.N., Macklaim, J.M., McMurrough, T.A., Edgell, D.R., Gloor, G.B., 2014. Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA gene sequencing and selective growth experiments by compositional data analysis. Microbiome 2, 15. https://doi.org/10.1186/2049-2618-2-15.

Flom St., M., John, A.M., Meyer, J.S., Tarullo, A.R., 2017. Infant hair cortisol: associations with salivary cortisol and environmental context. Dev. Psychobiol. 59, 26–38. https://doi.org/10.1002/dev.21449.

Fouhy, F., Watkins, C., Hill, C.J., O'Shea, C.-A., Nagle, B., Dempsey, E.M., O'Toole, P.W., Ross, R.P., Ryan, C.A., Stanton, C., 2019. Perinatal factors affect the gut microbiota up to four years after birth. Nat. Commun. 10, 1517. https://doi.org/10.1038/s41467-019-09252-4.

Gagliano, H., Delgado-Morales, R., Sanz-Garcia, A., Armario, A., 2014. High doses of the histone deacetylase inhibitor sodium butyrate trigger a stress-like response. Neuropharmacology 79, 75–82. https://doi.org/10.1016/j.neuropharm.2013.10.031.

Gray, N.A., Dhana, A., Van Der Vyver, L., Van Wyk, J., Khumalo, N.P., Stein, D.J., 2018. Determinants of hair cortisol concentration in children: a systematic review. Psychoneuroendocrinology 87, 204–214. https://doi.org/10.1016/j.psyneuen.2017.10.022.

Hamer, H.M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F.J., Brummer, R.-J., 2008. Review article: the role of butyrate on colonic function. Aliment. Pharmacol. Ther. 27, 104–119. https://doi.org/10.1111/j.1365-2036.2007.03562.x.

Holmberg, E., Kataja, E.-L., Davis, E.P., Pajulo, M., Nolvi, S., Hakanen, H., Karlsson, L., Karlsson, H., Korja, R., 2022. The connection and development of unpredictability and sensitivity in maternal care across early childhood. Front. Psychol. 13, 803047 https://doi.org/10.3389/fpsyg.2022.803047.

Hoyles, L., Snelling, T., Umlai, U.-K., Nicholson, J.K., Carding, S.R., Glen, R.C., McArthur, S., 2018. Microbiome–host systems interactions: protective effects of propionate upon the blood–brain barrier. Microbiome 6, 55. https://doi.org/10.1186/s40168-018-0439-y.

Hyppänen, H.-M., 2020. Early-life overweight and obesity in the FinnBrain Birth Cohort Study and Finland in 1- and 2-year-olds (Second cycle degree thesis). University of Turku.

Jahnke, J.R., Roach, J., Azcarate-Peril, M.A., Thompson, A.L., 2021. Maternal precarity and HPA axis functioning shape infant gut microbiota and HPA axis development in humans. PLoS ONE 16, e0251782. https://doi.org/10.1371/journal.pone.0251782.

Jašarević, E., Morrison, K.E., Bale, T.L., 2016. Sex differences in the gut microbiome–brain axis across the lifespan. Philos. Trans. R. Soc. B 371, 20150122. https://doi.org/10.1098/rstb.2015.0122.

Kamin, H.S., Kertes, D.A., 2017. Cortisol and DHEA in development and psychopathology. Horm. Behav. 89, 69–85. https://doi.org/10.1016/j.yhbeh.2016.11.018.

Karlsson, F.H., Fåk, F., Nookaew, I., Tremaroli, V., Fagerberg, B., Petranovic, D., Bäckhed, F., Nielsen, J., 2012. Symptomatic atherosclerosis is associated with an altered gut metagenome. Nat. Commun. 3, 1245. https://doi.org/10.1038/ncomms2266.

Karlsson, L., Tolvanen, M., Scheinin, N.M., Uusitupa, H.-M., Korja, R., Ekholm, E., Tuulari, J.J., Pajulo, M., Huotilainen, M., Paunio, T., Karlsson, H., FinnBrain Birth Cohort Study Group, 2018. Cohort profile: the finnbrain birth cohort study (FinnBrain). Int. J. Epidemiol. 47, 15–16j. https://doi.org/10.1093/ije/dyx173.

Keskitalo, A., Aatsinki, A.-K., Kortesluoma, S., Pelto, J., Korhonen, L., Lahti, L., Lukkarinen, M., Munukka, E., Karlsson, H., Karlsson, L., 2021. Gut microbiota diversity but not composition is related to saliva cortisol stress response at the age of 2.5 months. Stress 24, 551–560. https://doi.org/10.1080/10253890.2021.1895110.

Kische, H., Voss, C., Haring, R., Ollmann, T.M., Pieper, L., Kirschbaum, C., Beesdo-Baum, K., 2022. Hair androgen concentrations and depressive disorders in adolescents from the general population. Eur. Child Adolesc. Psychiatry. https://doi.org/10.1007/s00787-021-01929-w.

Kolde, R., 2019. pheatmap: Pretty Heatmaps. R package version 1.0.12.

Kong, X.-J., Liu, J., Liu, K., Koh, M., Sherman, H., Liu, S., Tian, R., Sukijthamapan, P., Wang, J., Fong, M., Xu, L., Clairmont, C., Jeong, M.-S., Li, A., Lopes, M., Hagan, V., Dutton, T., Chan, S.-T., (Phoebe), Lee, H., Kendall, A., Kwong, K., Song, Y., 2021. Probiotic and oxytocin combination therapy in patients with autism spectrum disorder: a randomized, double-blinded, placebo-controlled pilot trial. Nutrients 13, 1552. https://doi.org/10.3390/nu13051552.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.-M., Kennedy, S., Leonard, P., Li, J., Burgdorf, K., Grarup, N., Jørgensen, T., Brandslund, I., Nielsen, H.B., Juncker, A.S., Bertalan, M., Levenez, F., Pons, N., Rasmussen, S., Sunagawa, S., Tap, J., Tims, S., Zoetendal, E.G., Brunak, S., Clément, K., Doré, J., Kleerebezem, M., Kristiansen, K., Renault, P., Sicheritz-Ponten, T., de Vos, W.M., Zucker, J.-D., Raes, J., Hansen, T., MetaHIT consortium, Guedon, Delorme, E., Layec, C., Khaci, S., van de Guchte, G., Vandemeulebrouck, M., Jamet, G., Dervyn, A., Sanchez, R., Maguin, N., Haimet, E., Winogradski, F., Cultrone, Y., Leclerc, A., Juste, M., Blottière, C., Pelletier, H., LePaslier, E., Artiguenave, D., Bruls, F., Weissenbach, T., Turner, J., Parkhill, K., Antolin, J., Manichanh, M., Casellas, C., Boruel, F., Varela, N., Torrejon, E., Guarner, A., Denariaz, F., Derrien, G., van Hylckama Vlieg, M., Veiga, J.E.T., Oozeer, P., Knol, R., Rescigno, J., Brechot, M., M'Rini, C., Mérieux, C., Yamada, A., Bork, T., Wang, P., Ehrlich, J., Pedersen, S.D., O, 2013. Richness of human gut microbiome correlates with metabolic markers. Nature 500, 541–546. https://doi.org/10.1038/nature12506.

Lopetuso, L.R., Scaldaferri, F., Petito, V., Gasbarrini, A., 2013. Commensal clostridia: leading players in the maintenance of gut homeostasis. Gut Pathog. 5, 23. https://doi.org/10.1186/1757-4749-5-23.

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 1–21. https://doi.org/10.1186/s13059-014-0550-8.

Macutkiewicz, C., Carlson, G., Clark, E., Dobrindt, U., Roberts, I., Warhurst, G., 2008. Characterisation of Escherichia coli strains involved in transcytosis across gut epithelial cells exposed to metabolic and inflammatory stress. Microbes. Infect. 10, 424–431. https://doi.org/10.1016/j.micinf.2008.01.001.

Mann, C., Schloß, S., Cosan, A., Becker, K., Skoluda, N., Nater, U.M., Pauli-Pott, U., 2021. Hair cortisol concentration and neurocognitive functions in preschool children at risk of developing attention deficit hyperactivity disorder. Psychoneuroendocrinology 131, 105322. https://doi.org/10.1016/j.psyneuen.2021.105322.

Marceau, K., Wang, W., Robertson, O., Shirtcliff, E.A., 2020. A systematic review of hair cortisol during pregnancy: reference ranges and methodological considerations. Psychoneuroendocrinology 122, 104904. https://doi.org/10.1016/j.psyneuen.2020.104904.

McGuinness, A.J., Davis, J.A., Dawson, S.L., Loughman, A., Collier, F., O'Hely, M., Simpson, C.A., Green, J., Marx, W., Hair, C., Guest, G., Mohebbi, M., Berk, M., Stupart, D., Watters, D., Jacka, F.N., 2022. A systematic review of gut microbiota composition in observational studies of major depressive disorder, bipolar disorder and schizophrenia. Mol. Psychiatry 27, 1920–1935. https://doi.org/10.1038/s41380-022-01456-3.

Michels, N., Van de Wiele, T., Fouhy, F., O'Mahony, S., Clarke, G., Keane, J., 2019. Gut microbiome patterns depending on children's psychosocial stress: reports versus biomarkers. Brain Behav. Immun. 80, 751–762. https://doi.org/10.1016/j.bbi.2019.05.024.

Monsorno, K., Buckinx, A., Paolicelli, R.C., 2022. Microglial metabolic flexibility: emerging roles for lactate. Trends Endocrinol. Metab. 33, 186–195. https://doi.org/10.1016/j.tem.2021.12.001.

Morris, D.J., Brem, A.S., 2019. Role of gut metabolism of adrenal corticosteroids and hypertension: clues gut-cleansing antibiotics give us. Physiol. Genom. 51, 83–89. https://doi.org/10.1152/physiolgenomics.00115.2018.

Mustonen, P., Karlsson, L., Kataja, E.-L., Scheinin, N.M., Kortesluoma, S., Coimbra, B., Rodrigues, A.J., Sousa, N., Karlsson, H., 2019. Maternal prenatal hair cortisol is associated with prenatal depressive symptom trajectories. Psychoneuroendocrinology 109, 104383. https://doi.org/10.1016/j.psyneuen.2019.104383.

Nearing, J.T., Douglas, G.M., Hayes, M.G., MacDonald, J., Desai, D.K., Allward, N., Jones, C.M.A., Wright, R.J., Dhanani, A.S., Comeau, A.M., Langille, M.G.I., 2022. Microbiome differential abundance methods produce different results across 38 datasets. Nat. Commun. 13, 342. https://doi.org/10.1038/s41467-022-28034-z.

Nguyen, T.-V., Wu, M., Lew, J., Albaugh, M.D., Botteron, K.N., Hudziak, J.J., Fonov, V.S., Collins, D.L., Campbell, B.C., Booij, L., Herba, C., Monnier, P., Ducharme, S., McCracken, J.T., 2017. Dehydroepiandrosterone impacts working memory by shaping cortico-hippocampal structural covariance during development. Psychoneuroendocrinology 86, 110–121. https://doi.org/10.1016/j.psyneuen.2017.09.013.

Nikolova, V.L., Smith, M.R.B., Hall, L.J., Cleare, A.J., Stone, J.M., Young, A.H., 2021. Perturbations in gut microbiota composition in psychiatric disorders: a review and

meta-analysis. JAMA Psychiatry 78, 1343. https://doi.org/10.1001/jamapsychiatry.2021.2573.
Oluwagbemigun, K., Schnermann, M.E., Schmid, M., Cryan, J.F., Nöthlings, U., 2022. A prospective investigation into the association between the gut microbiome composition and cognitive performance among healthy young adults. Gut Pathog. 14, 15. https://doi.org/10.1186/s13099-022-00487-z.
Png, C.W., Lindén, S.K., Gilshenan, K.S., Zoetendal, E.G., McSweeney, C.S., Sly, L.I., McGuckin, M.A., Florin, T.H.J., 2010. Mucolytic bacteria with increased prevalence in ibd mucosa augment in vitro utilization of mucin by other bacteria. Am. J. Gastroenterol. 105, 2420–2428. https://doi.org/10.1038/ajg.2010.281.
Pyle Hennessey, E.-M., Kepinska, O., Haft, S.L., Chan, M., Sunshine, I., Jones, C., Hancock, R., Hoeft, F., 2020. Hair cortisol and dehydroepiandrosterone concentrations: associations with executive function in early childhood. Biol. Psychol. 155, 107946 https://doi.org/10.1016/j.biopsycho.2020.107946.
Rosin, S., Xia, K., Azcarate-Peril, M.A., Carlson, A.L., Propper, C.B., Thompson, A.L., Grewen, K., Knickmeyer, R.C., 2021. A preliminary study of gut microbiome variation and HPA axis reactivity in healthy infants. Psychoneuroendocrinology 124, 105046. https://doi.org/10.1016/j.psyneuen.2020.105046.
Roswall, J., Olsson, L.M., Kovatcheva-Datchary, P., Nilsson, S., Tremaroli, V., Simon, M.-C., Kiilerich, P., Akrami, R., Krämer, M., Uhlén, M., Gummesson, A., Kristiansen, K., Dahlgren, J., Bäckhed, F., 2021. Developmental trajectory of the healthy human gut microbiota during the first 5 years of life. Cell Host Microbe. 29 (e3), 765–776. https://doi.org/10.1016/j.chom.2021.02.021.
Russell, E., Kirschbaum, C., Laudenslager, M.L., Stalder, T., de Rijke, Y., 2015. Toward standardization of hair cortisol measurement: results of the first international interlaboratory round robin. Ther. Drug Monit. 37.
Russell, G., Lightman, S., 2019. The human stress response. Nat. Rev. Endocrinol. 15, 525–534. https://doi.org/10.1038/s41574-019-0228-0.
Saari, A., Sankilampi, U., Hannila, M.-L., Kiviniemi, V., Kesseli, K., Dunkel, L., 2011. New finnish growth references for children and adolescents aged 0 to 20 years: Length/height-for-age, weight-for-length/height, and body mass index-for-age. Ann. Med. 43, 235–248. https://doi.org/10.3109/07853890.2010.515603.
Scheiman, J., Luber, J.M., Chavkin, T.A., MacDonald, T., Tung, A., Pham, L.-D., Wibowo, M.C., Wurth, R.C., Punthambaker, S., Tierney, B.T., Yang, Z., Hattab, M.W., Avila-Pacheco, J., Clish, C.B., Lessard, S., Church, G.M., Kostic, A.D., 2019. Meta-omics analysis of elite athletes identifies a performance-enhancing microbe that functions via lactate metabolism. Nat. Med. 25, 1104–1109. https://doi.org/10.1038/s41591-019-0485-4.
Stalder, T., Kirschbaum, C., 2012. Analysis of cortisol in hair – state of the art and future directions. Brain Behav. Immun. 26, 1019–1029. https://doi.org/10.1016/j.bbi.2012.02.002.
Sudo, N., Chida, Y., Aiba, Y., Sonoda, J., Oyama, N., Yu, X.-N., Kubo, C., Koga, Y., 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice: commensal microbiota and stress response. J. Physiol. 558, 263–275. https://doi.org/10.1113/jphysiol.2004.063388.
Tomlinson, J.W., Stewart, P.M., 2001. Cortisol metabolism and the role of 11β-hydroxysteroid dehydrogenase. Best. Pract. Res. Clin. Endocrinol. Metab. 15, 61–78. https://doi.org/10.1053/beem.2000.0119.
van de Wouw, M., Boehme, M., Lyte, J.M., Wiley, N., Strain, C., O'Sullivan, O., Clarke, G., Stanton, C., Dinan, T.G., Cryan, J.F., 2018. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations: SCFAs alleviate stress-induced brain-gut axis alterations. J. Physiol. 596, 4923–4944. https://doi.org/10.1113/JP276431.
Vining, R.F., McGinley, R.A., Symons, R.G., 1983. Hormones in saliva: mode of entry and consequent implications for clinical interpretation. Clin. Chem. 29, 1752–1756. https://doi.org/10.1093/clinchem/29.10.1752.
Vital, M., Karch, A., Pieper, D.H., 2017. Colonic butyrate-producing communities in humans: an overview using omics data. mSystems 2 (e00130–17). https://doi.org/10.1128/mSystems.00130-17.
Wester, V.L., van der Wulp, N.R.P., Koper, J.W., de Rijke, Y.B., van Rossum, E.F.C., 2016. Hair cortisol and cortisone are decreased by natural sunlight. Psychoneuroendocrinology 72, 94–96. https://doi.org/10.1016/j.psyneuen.2016.06.016.
Wu, W.-L., Adame, M.D., Liou, C.-W., Barlow, J.T., Lai, T.-T., Sharon, G., Schretter, C.E., Needham, B.D., Wang, M.I., Tang, W., Ousey, J., Lin, Y.-Y., Yao, T.-H., Abdel-Haq, R., Beadle, K., Gradinaru, V., Ismagilov, R.F., Mazmanian, S.K., 2021. Microbiota regulate social behaviour via stress response neurons in the brain. Nature 595, 409–414. https://doi.org/10.1038/s41586-021-03669-y.