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Low salivary cortisol levels in infants of families with an anthroposophic lifestyle[☆]

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Summary The anthroposophic lifestyle implies environmental conditions for the infant aimed at reducing negative stress stimulation and is also related to a lower prevalence of allergic diseases in children. One aim of this prospective birth cohort study was to assess stress levels in infants with an anthroposophic lifestyle. A total of 330 infants from families with anthroposophic or more conventional lifestyles were followed from pregnancy of their mothers until the age of 6 months. Information on lifestyle factors was obtained from questionnaires. Salivary samples from 210 6-month olds and their parents were collected on three occasions during 1 day for analysis of cortisol. Infants from families with an anthroposophic lifestyle had significantly lower cortisol levels on all three sampling occasions compared to other infants. In the morning, the geometric means of salivary cortisol concentration (with 95% confidence limits) were 8.8 nmol/l (6.7–11.5), 11.3 nmol/l (9.3–13.7) and 14.9 nmol/l (11.3–19.6) in infants classified as anthroposophic, partly anthroposophic and non-anthroposophic, respectively ($p = 0.018$). On the other hand, there was no difference in cortisol levels between the parents in the different groups. Several lifestyle factors differed significantly between the groups, but none of them independently explained the difference in cortisol levels. However, living on a farm during pregnancy was significantly associated with low saliva cortisol level in the infant. It can be concluded that low salivary cortisol levels in infants from anthroposophic families may be related to an environment with a lower degree of exposure to stress, which could influence the development of allergic diseases.

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[☆] Please see Brief Commentary by Holger Ursin in this issue.

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1. Introduction

The prevalence of IgE-associated allergic diseases has increased markedly during the past decades, especially in children, although recent reports indicate that the occurrence in some regions has stabilised (Asher et al., 2006). Several factors, including an anthroposophic lifestyle, have been associated with protection against development of allergic diseases (Alm et al., 1999; Strachan, 2000; Garn and Renz, 2007). The protective effect of an anthroposophic lifestyle during early childhood has been attributed to factors like an organic diet including fermentation by live lactobacilli, home deliveries and restrictive use of antibiotics, anti-pyretics and vaccinations (Alm et al., 1999; Alfvén et al., 2006; Floistrup et al., 2006; Dicksved et al., 2007). However, an anthroposophic lifestyle also implies specific environmental conditions for the infant aimed at reducing negative stress (Glöcker and Goebel, 2007). A relationship between psychosocial factors like exposure to environmental stressors and the development of atopic disorders in children has been indicated (Chida et al., 2008). Further, parental stress has been associated to later development of asthmatic and allergic diseases in children (Milam et al., 2008; Cookson et al., 2009) and alterations in cortisol levels have been shown in children predisposed to disease (Ball et al., 2006). One possible pathway could be a “stress-modified” hypothalamus-pituitary-adrenal (HPA)-axis (Knackstedt et al., 2005).

Cortisol is the main end product of the HPA-axis activity and has a central role for several organ systems (Sapolsky et al., 2000). There is a close relationship between variations in serum/plasma concentration of free cortisol and saliva cortisol (Kirschbaum and Hellhammer, 1994). Saliva sampling has been applied in a large number of studies on the impact of environmental influences during infancy on behaviour and cognition, which support previous findings from animal studies (Lupien et al., 2009). To our knowledge no study has been published on cortisol levels in infants who grow up in an environment characterised by intentional stress regulation, such as in the anthroposophic society. The low risk of allergy among children with an anthroposophic lifestyle adds to the importance of such a study. Thus, the aim of our study was to investigate if infants in families with an anthroposophic lifestyle have different levels of salivary cortisol than other children.

2. Methods

This study is based on ALADDIN (Assessment of Lifestyle and Allergic Disease During Infancy), a prospective birth cohort study which started in 2004 and focuses on the impact of lifestyle during pregnancy and early childhood on the development of allergic disease. A total of 330 families were enrolled between September 2004 and November 2007. Families were recruited at anthroposophic health care centres in Järna ($n = 120$) and Stockholm ($n = 69$) and from conventional health care centres in Järna ($n = 121$) and the nearby town Södertälje ($n = 20$). All families from conventional maternal health care centres continued to attend conventional child health care centres in or close to Järna. Järna is a countryside village outside Stockholm with a large

community of anthroposophic followers. During pregnancy families were referred by their midwife at the maternal health care centre for written and oral information from the study coordinator. Of all families ($n = 201$) admitted to non-anthroposophic healthcare centres during the recruitment period 10 families did not get information from their midwife about the study and 25 declined further information. Another 25 of the families receiving information from the study coordinator declined participation, leaving 141 for inclusion. Corresponding numbers for anthroposophic health care centres ($n = 289$) were 20 families not getting information about the study from their midwife, 61 declining information from the study coordinator, 19 subsequently declining participation and 189 included. Families being positive to participation were included in the study in gestational weeks 28–32 and followed prospectively. Infants born before gestational week 36 and miscarriages were excluded from study participation.

Collection of information on lifestyle factors during pregnancy and delivery was based on previously used questionnaires (Alm et al., 1999), which were evaluated with parents and the study doctor when performing examinations of the infants at the age of 2 and 6 months. The degree of adherence to an anthroposophic lifestyle was categorised as “anthroposophic”, “partly anthroposophic” or “non-anthroposophic”, based on choice of health care centre and parental responses to three questions: (1) “What kind of preschool/school will your newborn child probably go to?” (2) “Has any of the parents, no matter which type of school you have planned for your child, an anthroposophic view of life?” and (3) “Is the family’s everyday life influenced by an anthroposophic view of life?” Families answering “anthroposophic school” to question 1 and “yes” to questions 2 and 3 and also attending anthroposophic health care centres were defined as “anthroposophic”. Families answering conventional type of school to question 1, “no” to questions 2 and 3 and going to conventional health care centre were defined as “non-anthroposophic”. Any other combination of answers was defined as “partly anthroposophic”.

Cortisol samples were obtained at home from the infant and both parents at the age of 6 months of the infant, in the morning, afternoon and evening. The morning sampling occasion was defined as “a quarter after awakening and before first meal”; afternoon as “after midday sleep” (or alternatively, if the child did not sleep, “one hour after midday meal”) in the infant group and as “before dinner or – if dinner was later – before 6 PM” in the parental group; and evening as “before going to bed”. The families also answered a short questionnaire regarding any unusual events or health problems during the day of sampling. The infants’ saliva samples were collected by sterile rolls (Braided cotton dental rolls; Richmond Dental, Charlotte, NC, USA), which the parents were instructed to keep in the mouth of the child for about 1–3 min, until soaked with saliva. Parental saliva samples were collected by swabs (Salivette; Sarstedt Inc., Rommelsdorf, Germany), which the participants were instructed to keep in their mouth until soaked with saliva. The samples were centrifuged, frozen at -70°C and stored until analyses were performed. All samples were analysed according to the manufacturer’s instructions, using the Spectria Cortisol RIA (125I) kit from Orion Diagnostica, Espoo, Finland (Hansen et al., 2003). Parent and infant samples from the same family were analysed in the same assay

Table 1 Number of individuals and saliva sampling times for infants and parents according to lifestyle group.

	Anthroposophic			Partly anthroposophic			Non-anthroposophic		
	Number	Mean (hour)	Std. dev. (h)	Number	Mean (h)	Std. dev. (h)	Number	Mean (h)	Std. dev. (h)
Infant									
Morning	52	7:37	±1:03	76	7:45	±1:05	70	7:40	±1:02
Afternoon	53	15:33	±1:49	77	15:06	±2:04	73	14:59	±1:44
Evening	50	19:58	±1:29	73	20:02	±1:30	71	19:46	±1:11
Mother									
Morning	49	7:20	±0:56	79	7:41	±1:04	71	7:34	±1:01
Afternoon	49	16:19	±1:39	78	16:53	±1:54	74	16:41	±1:12
Evening	47	22:03	±1:10	77	22:13	±1:24	73	21:40	±1:09
Father									
Morning	46	7:26	±1:11	76	7:33	±1:37	67	7:31	±1:10
Afternoon	47	16:06	±2:15	76	16:46	±2:13	68	16:41	±2:31
Evening	45	21:55	±1:45	73	22:30	±1:32	66	22:12	±1:03

and cortisol levels were expressed in nmol/l. The procedure for saliva collection has previously been validated and shown to be adequate (Stenius et al., 2008). Furthermore a comparison of the cortisol analyses for 30 saliva samples with a German laboratory at the Department of Psychology, Technical University of Dresden showed a high correlation (0.98) but systematically lower levels in the Stockholm laboratory. The difference was 12.5% with 95% confidence limits 1.5–22.3%.

SPSS version 17.0 was used for all statistical analyses. Chi-square analyses and ANOVA were used for comparison of lifestyle factors between the groups. ANOVA was used for comparing sampling times between the families. Since saliva cortisol showed a skewed distribution in all study groups and on all occasions, logarithmic transformation was used in the statistical computations. The log transformed saliva cortisol concentration was normally distributed. The explanatory variables on the other hand were category variables. However, the lifestyle variable showed a close to normal distribution (skewness -0.072) and linear regression was considered to be adequate. *P*-values less than 0.05 were regarded as statistically significant. Linear regressions were performed by a model using morning-, afternoon- and evening-values of the children's cortisol level as primary outcome.

The study was approved by the local Ethics Committee and written informed consent was obtained from all families.

3. Results

Of 330 families recruited 4 were excluded due to preterm delivery and 2 due to miscarriage. Nineteen families decided not to continue in the study: 6 due to stressful situations/complications during delivery, 4 due to diseases of the child, 3 due to moving, 1 because of disease of the mother during pregnancy, and 5 for unknown reasons.

At the age of 6 months of the infants 305 families remained in the study and of these 210 (53 anthroposophic, 82 partly anthroposophic and 75 non-anthroposophic) families collected salivary samples. Most of these families collected samples at all three time points (Table 1). The saliva sampling times for infants, mothers and fathers did not differ significantly between the groups.

Several prenatal, delivery and postnatal lifestyle factors differed significantly between the groups. Some of these characteristics of the three lifestyle groups are shown in Table 2. For example, 47.2% of the anthroposophic infants were born at home, compared to 22% of the partly anthroposophic and none of the non-anthroposophic infants. The 95 families not collecting salivary samples did not differ significantly from those collecting samples according to the anthroposophic lifestyle factors in Table 2 (data not shown).

The results of the salivary cortisol analyses for the infants, mothers and fathers are shown in Table 3. Infants of families with an anthroposophic lifestyle had significantly lower cortisol levels on all three sampling occasions compared to infants in the partly anthroposophic and non-anthroposophic groups. In particular, there seemed to be a lack of infants with high salivary cortisol levels in the anthroposophic group (Fig. 1). There was no significant difference in parental cortisol levels between the three groups, either in the mothers or fathers (Table 3). No differences were found between the groups in their answers to the short questionnaire regarding unusual events and health conditions during the day of saliva collection (data not shown).

In order to assess the contribution of certain lifestyle factors on the saliva cortisol level of the infants we performed a series of multiple linear regressions with the continuous variable morning cortisol (logarithmic values) as outcome and lifestyle group as main exposure factor. The independent contribution of the lifestyle factor on the cortisol levels of the infant was confirmed in all analyses. Also, in the corresponding analyses of afternoon and evening levels, the significant contribution of the lifestyle groups remained. In the model we successively analysed the effect of all exposure factors that significantly differed between the groups (Table 2). None of them could independently explain the lifestyle correlation. One factor, mother living on a farm during pregnancy, contributed significantly in the multiple regression analysis to morning cortisol level of the infants, but this was independent of lifestyle group. Thus, in multiple linear regression analysis both lifestyle group and living on a farm had independent significant effects on salivary cortisol with *p*-values of 0.010 and 0.019, respectively. Using back transformation of geometric means it was shown that among

Table 2 Examples of characteristics arranged according to lifestyle groups.

	Anthroposophic	Partly anthroposophic	Non-anthroposophic
Number of children (<i>n</i>)	53	82	75
Demographic			
Sex (% males)	49.1	47.6	48.0
Birth weight (kg, mean)	3.7	3.5	3.6
Birth height (cm, mean)	50.9	50.8	51
Age mother at birth (years, mean)	33.1	32.0	31.7
Age father at birth (years, mean)	36.6	36.0	34.9
Mother's reported rhinoconjunctivitis (%)	26.9	29.6	22.5
Father's reported rhinoconjunctivitis (%)	30.8	32.5	28.8
Living in apartment (%) [*]	43.4	47.6	17.3
Siblings or other children living with the family (<i>n</i>) [*]	1.09	0.77	0.76
Parental education gymnasium (% mother/% father)	41.5/37.8	37.8/40.7	46.7/55.4
Parental education university (% mother/% father)	37.7/35.8	52.4/45.7	41.3/32.4
Prenatal and delivery characteristics			
Organic a/o biodynamic diet during pregnancy (%) [*]	90.6	63.7	8.0
Use of butter on bread during pregnancy (%) [*]	43.4	18.3	1.3
Maternal smoking during pregnancy (%)	7.5	2.5	9.3
Living on a farm w animals during pregnancy (%) [*]	18.9	3.7	8.0
Delivery at home (%) [*]	47.2	22.0	0
Antibiotics during delivery (%)	11.3	4.9	8.2
Anaesthesia during delivery (%) [*]	28.3	49.4	89.0
Postnatal environmental characteristics			
Cow's milk formula first week (%)	13.5	13.4	27.0
First clothing of wool/silk (%) [*]	54.7	20.0	0
Breast-feeding at 6 months of age (%) [*]	96.2	90.2	75.3
AD-vitamins to the child (%) [*]	23.1	46.3	100
Colic during first 6 months (%)	11.8	7.4	19.2
First DTP-vaccination ^a before 6 months age (%) [*]	3.8	24.7	98.6
Pet animals at home at 6 months of age (%)	48.1	38.3	43.8
Living on a farm w animals at 6 months of age (%) [*]	17.3	4.9	4.1

^aDiphtheria, tetanus, pertussis.^{*} Differences significant with *p*-values <0.05).**Table 3** Salivary cortisol levels among infants, mothers and fathers, in three lifestyle groups in the morning, afternoon and evening. Geometric means (saliva cortisol, nmol/l) with 95% confidence intervals.

	Anthroposophic		Partly anthroposophic		Non-anthroposophic		<i>p</i> -value
	Geometric mean	Confidence intervals	Geometric mean	Confidence intervals	Geometric mean	Confidence intervals	
Infant							
Morning	8.8	6.7–11.5	11.3	9.3–13.7	14.9	11.3–19.6	0.018
Afternoon	3.0	2.2–4.2	5.5	4.4–6.8	6.8	5.0–9.3	0.001
Bedtime	1.9	1.4–2.7	2.7	2.1–3.6	4.2	2.9–6.0	0.006
Mother							
Morning	11.9	10.2–14.1	12.7	11.1–14.3	11.9	9.9–14.5	0.87
Afternoon	2.7	2.2–3.3	2.9	2.4–3.5	3.0	2.3–3.8	0.80
Bedtime	1.3	1.1–1.6	1.3	1.2–1.7	1.6	1.4–2.1	0.41
Father							
Morning	13.7	12.0–15.7	11.6	9.8–13.8	11.8	9.8–14.4	0.43
Afternoon	3.0	2.5–3.7	3.3	2.7–4.0	3.5	2.8–4.2	0.65
Bedtime	1.4	1.1–1.9	1.4	1.3–1.8	2.0	1.4–2.8	0.14

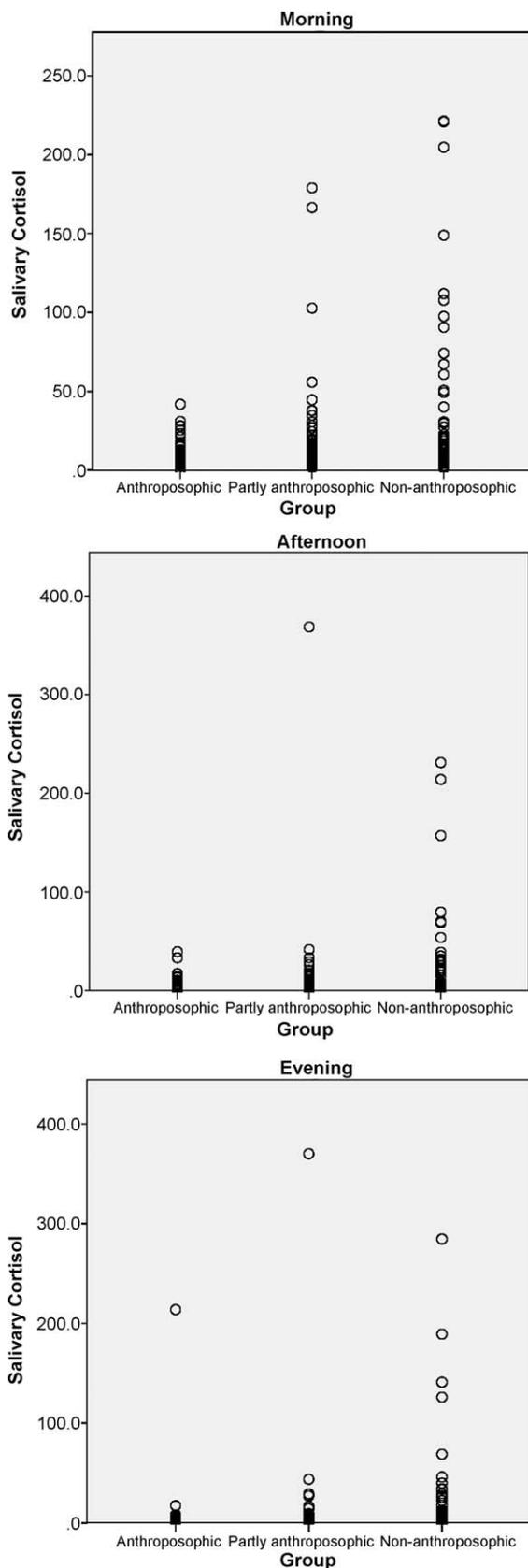


Figure 1 Salivary cortisol levels (nmol/l) of the children in the three groups (anthroposophic, partly anthroposophic and non-anthroposophic) on three occasions (morning, afternoon and evening).

infants growing up in the anthroposophic families and mother living on a farm during pregnancy reduced the salivary cortisol concentration by 41%. At the same time there was a 27% higher salivary cortisol concentration in the partly anthroposophic group than in the anthroposophic group and a 63% higher concentration in the non-anthroposophic compared to the anthroposophic group.

4. Discussion

In this study of salivary cortisol of 6-month olds and their parents, levels on all sampling occasions were lower in infants growing up in anthroposophic families than in families with more conventional lifestyle. Infants with the most anthroposophic lifestyle had the lowest levels of cortisol, those with the non-anthroposophic lifestyle the highest and the “partly anthroposophic group” levels in between, suggesting a “dose–response” relationship. Parental cortisol levels did not differ between lifestyle groups. Possibly, stress reducing environmental conditions related to the anthroposophic lifestyle may be more relevant for infants than for adults. The discrepancy between adult and infant levels may also reflect that early infancy is a period when certain stimuli may evoke cortisol reactions while such reactions will diminish or disappear over time (Egliston et al., 2007).

It could be argued that genetic variation may explain the differences between the infant study groups. However, the lack of differences concerning cortisol levels between the parents in the different study groups do not fit with such a hypothesis. Furthermore, the cortisol differences between infant lifestyle groups were least pronounced for the morning levels, usually regarded as more strongly genetically determined than levels collected during other parts of the day (Wust et al., 2000; Bartels et al., 2003).

There were several lifestyle factors that differed significantly between the groups, both pre- and postnatal, especially regarding circumstances around the delivery, clothing, dietary conditions and vaccinations. We made considerable efforts to find possible explanations for the findings. Mother living on a farm during pregnancy was the only exposure factor, beside the lifestyle group factor, that had significant influence on saliva cortisol concentration in the children. Prenatal exposure to farming has previously been associated with a lower risk of developing allergic diseases (Ege et al., 2008). However, this factor did not explain the association between anthroposophic group and salivary cortisol concentration in multivariate analysis.

The anthroposophic concept of the world advocates a holistic understanding of the human being as the basis for creating a society characterised by plurality and offering good developmental prerequisites for self-fulfilment. The anthroposophic community in Järna is integrated in society and is the third largest employer in a municipality (close to a metropolitan area) with 80,000 inhabitants. Compared to other anthroposophic communities in the world it has a more distinct geographic kernel with most of the important buildings in a rather small area. Even though this is situated in the countryside (and locally produced food stands out as another characteristic of this Swedish community) many of the individuals live in urban areas and work within “ordinary” professional branches. The community is highly interactive with other organisations, authorities and individuals by, for

instance, offering hospital and out patient care where conventional and complementary medicine are integrated, by running institutions for mentally handicapped children and adolescents, by making ecological food available in the whole country through two of the major national food suppliers and by contributing to the cultural life of the metropolitan area.

It is a difficult task – and an important focus for future research – to understand the environmental characteristics of this anthroposophic community which underlie the seemingly different regulation of cortisol in the infants. Our current main hypothesis is that anthroposophic families provide an infancy environment with a lower degree of exposure to everyday stressors. For instance, it is recommended for the infant to be protected from uncomfortable sensory stimulation such as strong light, unpleasant visual stimuli by choices of harmonious colours, and by scarcity of thoroughly selected toys (Glöcker and Goebel, 2007). A steady warm close environment is conveyed by use of clothes made by wool or silk. Sensual stimulation is carefully prepared for in many ways. The skin is stimulated not only by close body contact (use of a baby sling is common) but also by application of etheric oils (simultaneously creating pleasant olfactory stimulation). However, we have limited objective confirmation to what extent these precautions were practiced in the anthroposophic families under study, which means that our “sensory regulation hypothesis” needs to be verified in future empirical studies.

It also needs to be acknowledged that it is not self-evident that low cortisol levels imply good prerequisites for development and health. One may even speculate that they are indicators of understimulation. However, studies on deprivation – international adoptees that had been reared for more than 8 months in a Romanian orphanage – have demonstrated increased cortisol levels in comparison with non-adoptees (Gunnar et al., 2001). To the best of our knowledge there are no published long-term follow-up studies of the health during adult years in infants who were brought up in anthroposophic families. However there is one study on the health in adult years among children who went to a Steiner school associated with anthroposophic principles which showed a lower prevalence of hypertension and degenerative arthrosis during adulthood in the former Steiner school children (Barz and Randoll, 2007). This observation was not confounded by family income. Still, any conclusions about health effects related to the low cortisol levels in the anthroposophic children should be regarded as tentative.

It could be argued that our findings could be a result of the fact that many statistical significance tests were performed. However, the main finding was hypothesized in advance and indicated that there was a consistent graded difference in saliva cortisol concentration on all three sampling occasions during the day. With regard to possible competing explanatory factors we found no significant confounders despite the fact that detailed information was available on many lifestyle factors. All statistical analyses were performed with log normal transformations of the saliva cortisol concentrations. This was particularly important for the infant’s saliva cortisol concentrations since they were markedly skewed. The saliva cortisol concentrations in the parents were within normal levels compared with several other studies in our laboratory and also in comparison with most other published studies of normal adult populations (Alderling et al., 2006). One

strength of the study is that all the groups were fairly large and a comparatively high participation rate was obtained.

To conclude, we found an association between an anthroposophic lifestyle and low cortisol levels in infants, supporting the hypothesis of an anthroposophic environment characterised by a low degree of everyday stressors. Since an association between stress and the development of allergy has received certain empirical support, this low stress level may contribute to explaining the lower prevalence of allergic diseases in children with an anthroposophic lifestyle.

Conflicts of interest statements

We declare that we have no conflict of interests in connection with this paper.

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None.

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