

REGULAR ARTICLE

Lifestyle and salivary cortisol at the age of 12 and 24 months

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Keywords

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ABSTRACT

Aim: To analyse salivary cortisol levels in 12- and 24-month-olds from families with an anthroposophic lifestyle and comparisons ('partly anthroposophic' and 'non-anthroposophic').

Methods: Salivary samples were collected at child ages of 12 (n = 178) and 24 (n = 149) months. Cortisol was analysed with radioimmunoassay technique.

Results: Evening cortisol levels in children from anthroposophic families were lower than in comparisons at 12 months of age (geometric means: anthroposophic 1.7, partly anthroposophic 1.9, non-anthroposophic 3.6 nmol/L; p = 0.024) and at 24 months of age (1.1, 1.8 and 2.9 nmol/L, respectively; p = 0.002). At 24 months of age, similar differences were noted also for the afternoon levels (2.3, 3.3 and 3.9 nmol/L, respectively; p = 0.043). At age 12 months, the differences in the evening cortisol were statistically explained by a meat-free diet and at age 24 months by the anthroposophic lifestyle as such. The circadian variations were parallel in the three groups at age 12 and 24 months. No cortisol differences were observed between parents representing different lifestyles.

Conclusions: An anthroposophic lifestyle is associated with low cortisol levels in the evening at age 12 and 24 months, at age 24 months also in the afternoon.

BACKGROUND

In perceived stress, the HPA-axis (Hypothalamus-Pituitary-Adrenal cortex) is activated whereby its end product cortisol is released (1). There is a strong evidence that the functioning of the HPA-axis is influenced by early experiences (2,3). Both animal (4,5) and human studies (6–8) have demonstrated that this shaping of the axis starts already during the prenatal period. After birth environmental factors continue to influence the regulation of the HPA-axis. Several studies have shown that toddlers with a secure attachment do not exhibit elevations in cortisol, while those with an insecure relationship to their parent are much more likely to show increases (9–13). Neither anger nor fearfulness predicts increases in cortisol when the child is in the presence of an adult who is sensitive and responsive (10).

In a previous study, we have studied the effects of a lifestyle (anthroposophic) (14) with presumed health-promoting effects – as indicated for instance by lower prevalence of child allergy (15,16). The anthroposophic ideas of life involve a holistic view of life, including also spiritual values. For the Swedish general public, it is well known for its values concerning, for example, clothing (wool materials), a

diet of ecological/biodynamic products, emphasizing vegetarian components and scepticism about early vaccination. At 6 months of age infants from families with an anthroposophic lifestyle had significantly lower levels of salivary cortisol than comparisons (14). We tentatively interpreted these findings as expressions of certain potentially stress reducing elements of the anthroposophic lifestyle (17).

From several studies with different types of stressors, it becomes clear that cortisol reactivity to acute stressors decreases with age; weighing, examination, bathing episode, heel stick and vaccination provoke a small to moderate cortisol reaction in infants younger than 6 months but mostly smaller reaction in infants older than 6 months (18). Less is known about the development of the HPA-axis reactions to more complex and long-standing circumstances. A follow-

Abbreviations

HPA-axis, Hypothalamus-Pituitary-Adrenal cortex; ALADDIN, Assessment of Lifestyle and Allergic Disease During Infancy.

Key notes

- The lifestyle of parents may be associated with the cortisol levels of their infant.
- An anthroposophic lifestyle is associated with low cortisol levels in children at 6, 12 and 24 months of age, especially in the evening.
- A meat-free diet statistically explained the low cortisol levels at age 12 months in children from anthroposophic families.

up of anthroposophic children up to 12 and 24 months of age would open up for studies on such developmental processes. The socio-environmental environment is also continuously changing during this period with new challenges such as start of day care, usually during the beginning of the second year. Simultaneous parental sampling may give further clues to understand the interplay between the psychosocial environment and the HPA-axis regulation of the infants.

Accordingly, the aims of this study are as follows:

- 1 To investigate salivary cortisol levels over the day in 12- and 24-month-olds and their parents, comparing outcomes with regard to anthroposophic or conventional lifestyle.
- 2 To study how the cortisol levels develop over time (between ages of 6, 12 and 24 months).
- 3 To investigate whether any significantly different cortisol outcomes in the anthroposophic children can be explained by the factors studied in the present research design.

METHODS

Subjects

This study is based on Assessment of Lifestyle and Allergic Disease During INfancy (ALADDIN), a prospective birth cohort study focusing on the impact of lifestyle during pregnancy and early childhood. A total of 330 families were enrolled between September 2004 and November 2007. Of the 330 families recruited, four were excluded because of preterm delivery and two because of miscarriage. There was a gradually increasing attrition from the primary groups of 302 families who entered the study at the birth of their child with 52% ($n = 43$) with salivary samples remaining in the anthroposophic group at 24 months of child age, 41% ($n = 49$) in the partly anthroposophic group and 57% ($n = 57$) in the non-anthroposophic group.

The whole recruitment procedure has been described previously (14,19).

Degree of adherence to an anthroposophic lifestyle

The degree of adherence to an anthroposophic lifestyle was categorized as 'anthroposophic', 'partly anthroposophic' or 'non-anthroposophic', based upon the choice of antenatal clinic 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 question 2 and 3 and also attending anthroposophic antenatal clinics were defined as 'anthroposophic'. Families answering conventional or any other non-anthroposophic type of school to question 1, 'no' to question 2 and 3 and going to conventional antenatal clinic were defined as

'non-anthroposophic'. Any other combination of answers was defined as 'partly anthroposophic'.

Family characteristics

Several characteristics differed significantly between the lifestyle groups at age 12 and 24 months (Table S1). There were no significant differences – for any lifestyle group – between those who collected saliva at age 12 months (178 families: 47 anthroposophic, 65 partly anthroposophic and 66 non-anthroposophic) and those who did not (124 families: 36 anthroposophic, 54 partly anthroposophic and 34 non-anthroposophic). The situation was the same at age 24 months between those who collected saliva (149 families: 43 anthroposophic, 49 partly anthroposophic and 57 non-anthroposophic) and those who did not (153 families: 40 anthroposophic, 70 partly anthroposophic and 43 non-anthroposophic).

Cortisol analysis

Salivary cortisol samples were collected at home from the infant and both parents when child was 12 and 24 months old, in the morning, afternoon and evening (14). All samples were analysed according to the manufacturer's instructions, using the Spectria Cortisol RIA (125I) kit from Orion Diagnostica, Espoo, Finland. Parent and infant samples from the same family were always analysed in the same assay and cortisol levels were expressed in nmol/L. The procedure for salivary collection has been validated and shown to be adequate (20).

The numbers of salivary samples for each sampling time and lifestyle group are presented in Table 1 for children and parents, with reference to each child sampling age. The loss of samples for the analysis due to too little saliva increased with age 6, 12 and 24 months (21, 39 and 109, respectively), with an even distribution between day sampling times.

The families also filled in short questionnaires regarding any extraordinary events or health problems during the day of sampling: Questions regarding the child: 1) This day the child felt well (yes or no). 2) This day was a usual day for the child concerning sleep, food, and daily occurrences (yes or no). Questions regarding the parents: A) This day I felt well (yes or no). B) This day was a usual day concerning stress and daily hassles (yes or no). C) I felt well-rested after those hours (yes or no).

Statistical analysis

JMP 9.0 and SPSS Statistics 19.0 were used for the statistical analyses. As salivary cortisol showed a skewed distribution in all studied groups and on all occasions logarithmic transformation was used. Means are presented after back-transformation (geometric means). The corresponding 95% confidence limits are presented. 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. In addition two-way ANOVA was used for the study of circadian variation interacting with lifestyle group, with results indicating main effects of

Table 1 Salivary cortisol levels at 6, 12 and 24 months of child age in infants, mothers and fathers in the three groups on all sampling occasions (morning, afternoon and evening) with p-values corresponding to linear regressions with anthroposophic lifestyle group as explanatory and ln (cortisol concentration) as outcome variable. Geometric means (saliva cortisol, nmol/L) with confidence intervals and number of participants. (Data on 6-month-olds previously published Stenius et al. [15])

Months of age	Morning			Afternoon			Bedtime		
	6	12	24	6	12	24	6	12	24
	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean	Geometric mean
	Confidence interval	Confidence interval	Confidence interval	Confidence interval	Confidence interval	Confidence interval	Confidence interval	Confidence interval	Confidence interval
	Number	Number	Number	Number	Number	Number	Number	Number	Number
Infant									
Anthroposophic	8.8 6.7–11.5 (52)	7.5 5.4–10.4 (41)	6.0 3.6–10.0 (28)	3.0 2.2–4.2 (53)	3.5 2.6–4.7 (38)	2.3 1.6–3.4 (29)	1.9 1.4–2.7 (50)	1.7 1.3–2.3 (33)	1.1 0.8–1.3 (29)
Partly anthroposophic	11.3 9.3–13.7 (76)	9.0 6.5–12.4 (60)	10.7 7.4–15.4 (37)	5.5 4.4–6.8 (77)	3.1 2.1–4.5 (57)	3.3 2.3–4.8 (35)	2.7 2.1–3.6 (73)	1.9 1.2–3.0 (53)	1.8 1.2–2.9 (36)
Non-anthroposophic	14.9 11.3–19.6 (70)	10.9 7.9–14.8 (57)	8.8 6.4–12.1 (37)	6.8 5.0–9.3 (73)	4.9 3.5–6.9 (57)	3.9 2.9–5.4 (39)	4.2 2.9–6.0 (71)	3.6 2.3–5.6 (52)	2.9 1.8–4.8 (37)
p-value	0.018	0.119	0.198	0.001	0.143	0.043	0.006	0.024	0.002
Mother									
Anthroposophic	11.9 10.2–14.1 (49)	10.5 7.9–13.8 (45)	10.3 7.8–13.6 (42)	2.7 2.2–3.3 (49)	2.2 1.6–3.0 (41)	2.5 1.9–3.1 (40)	1.3 1.1–1.6 (47)	0.9 0.7–1.3 (42)	1.3 1.0–1.7 (38)
Partly anthroposophic	12.7 11.1–14.3 (79)	9.6 7.6–12.1 (61)	11.6 8.5–15.9 (43)	2.9 2.4–3.5 (78)	2.2 1.7–2.8 (60)	2.6 2.0–3.3 (45)	1.3 1.2–1.7 (77)	1.2 0.8–1.7 (55)	1.7 1.2–2.3 (44)
Non-anthroposophic	11.9 9.9–14.5 (71)	11.4 9.5–13.6 (64)	12.6 10.8–15.2 (53)	3.0 2.3–3.8 (74)	2.9 2.3–3.6 (64)	2.5 2.0–3.3 (54)	1.6 1.4–2.1 (73)	1.5 1.1–2.2 (62)	1.6 1.2–2.1 (53)
p-value	0.87	0.541	0.443	0.80	0.144	0.958	0.41	0.054	0.525
Father									
Anthroposophic	13.7 12.0–15.7 (46)	12.7 10.5–15.3 (42)	10.8 7.9–14.8 (37)	3.0 2.5–3.7 (47)	2.7 2.1–3.5 (40)	3.0 2.4–3.8 (38)	1.4 1.1–1.9 (45)	0.9 0.6–1.3 (38)	1.4 1.0–1.9 (33)
Partly anthroposophic	11.6 9.8–13.8 (76)	11.4 9.6–13.2 (55)	10.4 8.6–12.6 (44)	3.3 2.7–4.0 (76)	2.3 1.8–2.8 (55)	2.7 2.2–3.4 (45)	1.4 1.3–1.8 (73)	1.1 0.8–1.5 (52)	1.4 1.1–1.8 (43)
Non-anthroposophic	11.8 9.8–14.4 (67)	12.8 10.9–14.9 (61)	12.9 10.8–15.4 (50)	3.5 2.8–4.2 (68)	3.4 2.7–4.1 (62)	2.7 2.2–3.4 (51)	2.0 1.4–2.8 (66)	1.3 0.9–1.7 (60)	1.4 1.1–1.8 (51)
p-value	0.43	0.858	0.321	0.65	0.121	0.767	0.14	0.142	0.990

lifestyle, of time of day and of interaction between time of day and lifestyle group. These analyses were performed separately for infant age 6, 12 and 24 months. p-values <0.05 were considered statistically significant. The lifestyle variable had three categories and was close to normally distributed and therefore simple linear regression computations were performed using morning, afternoon and evening values of the children as well as their parent's salivary cortisol levels as separate primary outcomes and lifestyle group as explanatory variable.

The study was approved by the local Ethics Committee in Stockholm: (2002-01-07, Dnr 474/01, HS) and written informed consent was obtained from all families.

RESULTS

The salivary sampling times for infants, mothers and fathers did not differ significantly between the three lifestyle groups (Table S2). The only differences related to sampling days were lower frequencies of reports of stressful days or not feeling well-rested in the non-anthroposophic group at both 12 and 24 months (Table S3).

Evening salivary cortisol levels in children reared in anthroposophic families were lower than in comparisons at 12 months (Geometric means: anthroposophic 1.7, partly anthroposophic 1.9, non-anthroposophic 3.6 nmol/L; $p = 0.024$) and at 24 months of age (1.1, 1.8 and 2.9 nmol/L, respectively; $p = 0.002$). At 24 months of age, similar

differences were noted also for the afternoon levels (2.3, 3.3 and 3.9 nmol/L, respectively; $p = 0.043$) (Table 1). Looking at the cortisol levels at 6, 12 and 24 months together in the same model, there were lower levels in the anthroposophic children in the afternoon ($p = 0.025$) and in the evening ($p = 0.017$). No cortisol differences were observed between mothers or fathers representing different lifestyles.

In the total group of children, the salivary cortisol levels decreased on almost all sampling occasions between 6 and 12 months of age (morning $p = 0.005$; afternoon $p < 0.001$; bedtime $p = 0.035$) as well as between 12 and 24 months of age (morning $p = 0.022$; afternoon $p = 0.002$; bedtime $p = 0.015$). There were no interactions between sampling age and group meaning that the changes over time (6, 12 and 24 months) were parallel in all lifestyle groups.

Two-way analyses for the effects of circadian variation in the infants showed significant main effects of lifestyle group in all the studied ages ($F = 11.47$, $df = 1/178$ $p = 0.001$ at age 6 months, $F = 4.14$, $df = 1/120$ $p = 0.038$ at age 12 months and $F = 7.10$, $df = 1/72$, $p = 0.010$ at age 24 months). As expected there were also strong main effects of time of day but no significant interaction effects which mean that despite differences in saliva cortisol concentration between the groups the circadian patterns were parallel.

Multiple linear regression analyses were performed to examine potential explanatory factors of the low cortisol levels in the anthroposophic children in the evening at 12 months. In these analyses, lifestyle group was competing statistically with all factors that in univariate analyses both differed between lifestyle groups and were associated with low cortisol levels at 12 months: no vaccination during first 12 months ($p = 0.005$ at bedtime, $p = 0.039$ in the afternoon and not significant in the morning); meat-free diet ($p = 0.004$ at bedtime, not significant neither in the afternoon nor in the morning); still breast-feeding ($p = 0.012$ at bedtime, not significant neither in the afternoon nor in the morning). At 6 months of age, anthroposophic lifestyle was the only explanatory factor (14), but at the age of 12 months one of these lifestyle factors – a meat-free diet – explained the low levels in the anthroposophic children ($p = 0.048$).

At 24 months of age, the analyses of the same kind were performed. The univariate analyses showed that the evening salivary cortisol concentration was associated with meat-free diet ($p = 0.005$). There were also associations in the afternoon with meat-free diet ($p = 0.011$), fish-free diet ($p = 0.033$) and organic/biodynamic diet ($p = 0.003$). In the multivariate analyses, the evening levels were explained only by the anthroposophic lifestyle as such ($p = 0.022$). There was no explanation for the differences in the afternoon. See Figure S1 showing the distribution in the three groups of lifestyle factors (%) correlated (in univariate analyses in morning, afternoon or evening samples) with salivary levels of cortisol at child age 12 and 24 months.

DISCUSSION

Children reared in anthroposophic families had lower evening cortisol levels than comparisons at 12 and 24 months of

age and lower afternoon levels at 24 months of age. The circadian patterns changed similarly with gradually lower levels in all lifestyle groups between 6, 12 and 24 months. No cortisol differences were observed between mothers or fathers representing different lifestyles. In multiple linear regression analyses, meat-free diet significantly explained the low evening levels in the anthroposophic children at 12 months of age, whereas the low evening levels at 24 months of age were explained only by the anthroposophic lifestyle as such.

The differences in levels of cortisol between the infants are most evident at the age of 6 months. This could indicate that the environment of the anthroposophic families early in life is differently adapted to the nature of the infant with lower exposure to unpleasant perceptual stimuli (14). The fewer significant differences at 12 and 24 months of age suggest either that the environmental prerequisites were more similar between the groups at that stage or that the HPA-axis was to a lower extent involved in the adaptation of uncomfortable trivial stimuli at 12 months of age. The latter hypothesis would be congruent with previous findings that acute stressors are more likely to provoke cortisol reactions mostly in infants younger than 6 months (18).

One alternative hypothesis is that the differences between groups were because of genetically determined differences concerning diurnal cortisol levels. However, one would in that case have expected different cortisol levels also between parental groups. The absence of such differences gives some support to the hypothesis that the differences between child groups were related to environmental differences.

The influence of meat-free diet on levels of cortisol – supported by the multiple linear regression analyses at 12 months of age – was unexpected but is in line with results indicating that diet can have effects on adrenocortical activity with sustained endocrine changes. A high protein diet may increase cortisol levels compared to a high carbohydrate diet with parallel changes in corticosteroid binding globulin concentrations (21). Along the same line, a lacto vegetarian diet with low protein may reduce adrenocortical activity (22). There is evidence that an unbalanced high-meat/fish, low-green-vegetable diet during pregnancy may present a metabolic stress to the mother and programme the HPA-axis of the offspring, leading to lifelong hypercortisolemia (23). An unbalanced high protein maternal diet during late pregnancy is associated with increased cortisol secretion in response to psychological stress in the offspring (24). The influence of meat-free diet on levels of cortisol is interesting and motivates further investigations.

The anthroposophic ideas of life may seem challenging but obviously the lifestyle that is emerging as one of its consequences has the potential of influencing vital physiological systems. One may claim that this does not necessarily imply healthy consequences (25) even if low levels of cortisol are usually interpreted as a positive outcome (9–13). However, in a related study, we have also demonstrated that (high) cortisol levels at 6 months of age predict allergic manifestations at 2 years of age (26). This is especially

interesting given the low allergy incidence previously reported in anthroposophic children, a finding that has inspired to study this group more closely (15,16). Thus, we suggest that the anthroposophic environment may have a health-promoting influence, at least partly mediated via the HPA-axis. It is of general interest to identify the factor/factors or combination of factors that contribute to these low levels of cortisol. Hopefully, such a more deep-going analysis may bring out knowledge about environmental qualities that may be integrated into other types of lifestyles than the anthroposophic. The link between a meat-free diet and low cortisol levels is a finding that can illustrate such a work. The finding in itself needs to be replicated and studied more in detail before anything can be said about its relation to health but it may in the future bring out clues to a healthy diet. From such perspectives, more studies of the anthroposophic lifestyle could contribute to new knowledge of importance even for public health.

LIMITATIONS

A large number of factors may influence cortisol levels and it is not certain that we have selected neither the most important ones nor chosen the optimal way of measuring them. Similarly, there are a large number of factors – such as childhood adversities of various kinds – that we have no information about. Thus, our findings should be regarded as tentative. Still, we made an effort to capture any effects of a rather large number of factors that could be considered relevant given what is known about the anthroposophic community. We also tried to adapt the analyses for special circumstances on the sampling days by analysing parental questionnaires about the days in focus. These analyses demonstrated lower frequencies of reports of stressful days or not feeling well-rested in the non-anthroposophic group (Table S3). If such differences would have had any effects upon the cortisol levels, they would rather have attenuated the reported group differences.

It should be acknowledged that the attrition rate gradually increased, a common complication in prospective studies of this kind, implying that a selection bias cannot be ruled out.

From the primary groups of 302 families who entered the study at the birth of their child 52% (n = 43) with salivary samples remained in the anthroposophic group at 24 months of child age, 41% (n = 49) in the partly anthroposophic group and 57% (n = 57) in the non-anthroposophic group.

Notably, the attrition was quite similar in the two contrast groups. Furthermore, there were no differences concerning any of the characteristics of daily life for any lifestyle group between those who took part in the salivary sampling and those who did not.

The information about lifestyle is based mainly on parental reports. Cortisol data were based on the samplings from one single day, which should be taken into consideration when conclusions are drawn. The strengths of our study include the prospective design with families

recruited already during pregnancy and data collected longitudinally.

CONCLUSIONS

An anthroposophic lifestyle is associated with low evening cortisol levels in 12- and 24-month-olds and in afternoon cortisol at age 24 months. Meat-free diet in the children statistically explained the low levels at age 12 months. No cortisol differences were observed between parents representing different lifestyles.

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CONFLICTS OF INTEREST

Jackie Swartz is involved in several ways in the anthroposophic community.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Distribution in the three groups of life style factors (%) correlated (in univariate analyses in morning, afternoon or bedtime samples) with salivary levels of cortisol at child age 12 and 24 months.

Table S1 Demographic data and family characteristics for infants at 24 months of age arranged according to an anthroposophic, partly anthroposophic or non-anthroposophic life style. Categorical variables: n/N (%). Continuous variables: Mean \pm SD.

Table S2 Salivary samplings at 12 and 24 months of age. Number of individuals; mean saliva sampling times for infants and parents arranged according to an anthroposophic, partly anthroposophic or non-anthroposophic life style; standard deviation for sampling time.

Table S3 Questionnaires regarding any extraordinary events or health problems during the day of sampling for infants at 12 and 24 months of age, mothers and fathers arranged according to an anthroposophic, partly anthroposophic or non-anthroposophic life style; n/N (%). p-values.

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