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Basal or stress-induced cortisol and asthma development. The TRAILS study.

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Methods

Selection of adolescents performing a stress test

The laboratory tasks started between 0800h and 0930h (morning sessions, 49%) and 0100h and 0230h (afternoon sessions, 51%). The costly and labor-intensive nature of the experimental session precluded assessing the whole sample. To increase the power to detect mental health-related differences in the stress response, adolescents with a high risk of mental health problems had a greater chance of being selected for the laboratory tasks session compared to healthy adolescents. High risk was defined based on three criteria: 1. temperament assessed by the revised parental version of the Early Adolescent Temperament Questionnaire, (EATQ; i.e., high scores on frustration ($\geq 90^{th}$ percentile of EATQ), and fearfulness ($\geq 90^{th}$ percentile of EATQ), low scores on effortful control ($\leq 10^{th}$ percentile of EATQ)), 2. lifetime parental psychopathology (at least one parent with depression, anxiety, addiction, psychosis, or antisocial behavior), and 3. environmental risk (at least one of the biological parents was raised in a single-parent family), all assessed at age 11 years. In total, 66% had at least one of the above-described risk factors. The remaining 34% were randomly selected from the total TRAILS cohort. Please note that the focus sample still represented the whole range of problems seen in a normal population of adolescents, which made it possible to reproduce the distribution in the total TRAILS sample by means of sampling weights.

The study protocol was approved by the Central Committee on Research Involving Human Participants (CCMO). Participants provided written informed consent.

Forty-two (6%) adolescents had asthma, and 34 (5%) adolescents used corticosteroid-containing medication.

Stress task

The stress test consisted of two parts. In the first part, the adolescents were instructed to prepare a 6-minute speech about themselves and their lives and deliver this speech in front of a video camera. They were told that their videotaped performance would be judged by a panel of peers after the experiment. The adolescents had to speak continuously for the whole period of 6 minutes. The test assistant watched the performance critically, and showed no empathy or encouragement. The speech was followed by a 3-minute interlude in which the adolescents were not allowed to speak. During this interval, the adolescents were told that they had to wait for a moment because of computer problems, but that the task would continue as soon as the problems were solved. In the second part, adolescents were asked to perform a mental arithmic task. The adolescents were instructed to repeatedly subtract the number 17 from a larger sum, starting with 13,287. This difficult task was meant to induce a sense of uncontrollability. Uncontrollability was further provoked by negative feedback by the test assistant, including remarks such as, ‘‘No, wrong again, begin at 13,278’’, ‘‘Stop wiggling your hands’’ or ‘‘You are too slow, be as quick as you can, we are running out of schedule’’. The mental arithmetic challenge lasted for 6 minutes, again followed by a 3-minute period of silence, after which the adolescents were debriefed about the experiment.

Statistical analysis

With respect to cortisol measured upon awakening at age 11 years, the AUCg (11 years) was calculated using the formula (Cort7.30AM – Cort7.00AM)* 0.5 / 2 + 0.5 * Cort7.00AM. The AUCi (11 years) was calculated using the formula (Cort7.30AM – Cort7.00AM) * 0.5 / 2.

With respect to cortisol measured during the stress test, the AUCg (stress-induced) was calculated using the formula: (Cort1 + Cort2)* 25/2 + (Cort2 + Cort3)* 20/2 + (Cort3 + Cort4)*20/2. The AUCi (stress-induced) was calculated using the formula (Cort1 + Cort2)* 25/2 + (Cort2 + Cort3)* 20/2 + (Cort3 + Cort4)*20/2 – Cort1*65.

Search strategy for the meta-analysis

Relevant articles were identified by searching the database of Medline. The following search string was used to identify articles: the first component consisted of asthma; the second component consisted of the terms hypothalamic-pituitary-adrenal axis, pituitary-adrenal and cortisol; the third component consisted of the terms human, children, adolescents, childhood, adulthood, adult, adults.

Extraction of cortisol data

Morning and evening mean cortisol levels and standard deviations from asthmatics and non-asthmatics were extracted from the case-control studies or in case of missing information where calculated for each study. When cortisol levels were divided in nocturnal and non-nocturnal asthma or in mild and moderate-to-severe asthma, a pooled mean cortisol level and a pooled standard deviation was calculated. One study was excluded from the meta-analysis because calculation of mean cortisol levels and standard deviation in asthmatics and non-asthmatics was not possible. Relevant articles were identified by searching the database of Medline (published between 2012 and 1985). The following search string was used to identify articles: the first component consisted of asthma; the second component consisted of the terms hypothalamic-pituitary-adrenal axis, pituitary-adrenal and cortisol; the third component consisted of the terms human, children, adolescents, childhood, adulthood, adult, adults. This resulted in 601 articles. Title and abstract of relevant articles were screened based on: 1) case-control studies; 2) measurement of asthma; 3) measurement of HPA-axis. Articles written in another language than English, articles studying the effect of treatment on HPA-axis function, articles studying HPA-axis under provocation tests or stress (test), and clinical trials were

excluded. Full text of 11 articles was acquired. In case of suspected duplicate reports of the same subjects the original authors were contacted to detect overlap of individuals. This resulted in the exclusion of 1 article. In addition, 1 article was excluded because no basal cortisol levels could be calculated from the information provided in the study.

Statistical software used for pooling the data

Meta-analyst (http://www.medepi.net/meta/MetaAnalyst.html) was used for pooling of the data. The present of heterogeneity was calculated by the Q statistic (Chi-square test calculating whether variation in study results is due to chance variation or whether variation is due to systematic underlying differences and the null hypothesis should be rejected) and the I^2 statistic (the percentage of variability in the results that is caused by heterogeneity rather than coincidence).

Allergic and non-allergic asthma

Parentally reported information about allergy, hay fever and eczema was assessed at age 11, 14 and 16 years. Allergic asthma was defined as having asthma at a specific survey with allergy, hay fever or eczema at that survey. Non-allergic asthma was defined as having asthma at a specific survey without allergy, hay fever or eczema at that survey.

Logistic regression models were used to study the association of basal cortisol with allergic and non-allergic asthma, adjusted for sex and quadratic effect of sampling month. Logistic regression models were used to study the association of stress-induced cortisol with allergic and non-allergic asthma, adjusted for sex and sampling weights to correct for the

oversampling on high risk of mental health problems in case of AUCg (stress test) and additionally for baseline cortisol level (Cort1) in case of AUCi (stress test).

Table E1: Overview of studies included in the meta-analysis comparing standardized mean differences of morning cortisol levels

Study	Saliva/Serum	Cases			Controls		
		N	Mean (ng/ml)	SD (ng/ml)	N	Mean (ng/ml)	SD (ng/ml)
Fei 2004	Saliva	21	4.0	0.8	15	9.1	1.0
Bakkeheim 2010	Saliva	50	3.1	1.7	52	3.9	1.6
Buske-Kirschbaum 2003	Saliva	17	7.8	3.0	18	7.7	3.1
Nomura 1997	Serum	35	46.2	19.3	39	56.4	25.0
Haen 1991	Serum	10	250.1	103.3	8	206.6	30.8
Sutherland 2003	Serum	20	155.3	37.7	11	107.0	39.8
Griese 1988	Serum	21	126.9	77.7	7	132.5	55.8
Landstra 2002	Serum	28	116.0	86.3	18	159.5	92.3

Kallenbach 1988	Serum	13	148.5	39.8	11	151.8	32.5

N = number, SD = standard deviation

Table E2: Overview of studies included in the meta-analysis comparing standardized mean differences of evening cortisol levels

Study	Saliva/Serum	Cases			Controls		
		N	Mean (ng/ml)	SD (ng/ml)	N	Mean (ng/ml)	SD (ng/ml)
Fei 2004	Saliva	21	2.9	0.6	15	3.5	0.7
Bakkeheim 2010	Saliva	50	0.3	0.1	52	0.3	0.2
Haen 1991	Serum	10	54.4	34.4	8	32.6	41.0
Landstra 2002	Serum	28	32.6	95.9	18	38.1	69.2

N = number, SD = standard deviation

Figure E1: Funnel plot of studies investigating morning cortisol levels in asthmatics and non-asthmatics

Figure E2: Funnel plot of studies investigating evening cortisol levels in asthmatics and non-asthmatic