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

Nienke M. Vink, MD\textsuperscript{1,4}, H. Marike Boezen, PhD\textsuperscript{1,4}, Dirkje S. Postma, MD, PhD\textsuperscript{2,4}, Judith G.M. Rosmalen, PhD\textsuperscript{3}

\textsuperscript{1} Department of Epidemiology, University of Groningen, University Medical Center Groningen, Groninger Research Institute for Asthma and COPD, Groningen, The Netherlands

\textsuperscript{2} Department of Pulmonology, University of Groningen, University Medical Center Groningen, Groninger Research Institute for Asthma and COPD, Groningen, The Netherlands

\textsuperscript{3} Interdisciplinary Center for Psychiatric Epidemiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

\textsuperscript{4} GRIAC research institute, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

\textbf{Corresponding author:}

JGM Rosmalen, PhD

Interdisciplinary Center for Psychiatric Epidemiology

University of Groningen

University Medical Center Groningen

Hanzeplein 1
Running title:

Cortisol and asthma

Total word count:

Word count manuscript: 2944

Word count abstract: 198

Key words: asthma, cortisol, hypothalamic-pituitary-adrenal-axis, population cohort, adolescents
Abstract:

We examined the association between 1) cortisol levels and asthma or asthma development; 2) cortisol levels upon stress and asthma. In addition, we performed a post hoc meta-analysis on results from the literature.

Cortisol, cortisol upon stress, asthma (doctor diagnosis of asthma and/or symptoms and/or treatment in the past 12 months) and asthma development (asthma at a specific survey while not having asthma at the previous survey(s)) were assessed in the TRAILS study (n=2,230, mean age at surveys 1 (11 years), 2 (14 years), 3 (16 years)). Logistic regression models were used to study associations between 1) cortisol (cortisol awaking response (AUCg or AUCi) and evening cortisol (Cort8.00PM)) and asthma or asthma development; 2) cortisol upon stress (AUCg or AUCi) and asthma. The meta-analyses included 9 case-control articles on basal cortisol in asthma.

No significant association was found between 1) cortisol and asthma (age 11 years) or asthma development (age 14 or 16 years); 2) cortisol upon stress and asthma (age 16 years). The meta-analysis found lower morning and evening cortisol levels in asthmatics compared to non-asthmatics, however, the summary estimates were not significant.

We found no evidence supporting a role for cortisol in asthma and asthma development.
Introduction

Asthma is a chronic inflammatory airway disorder, with many pathways involved in its etiology. Psychosocial stress can trigger asthma exacerbations [1] and it is suggested that psychosocial stress is also involved in asthma development [2-6]. Psychosocial stress activates the hypothalamic-pituitary-adrenal (HPA) axis leading to an increase in cortisol secretion on top of the circadian rhythm of cortisol secretion [7]. Cortisol influences the activity of many systems in the human body, amongst which the immune system [8]. Since cortisol induces a shift in the Th1/Th2 balance of peripheral blood mononuclear cells towards a predominant Th2 response [7], an alteration in HPA-axis function is suggested to be one of the potential mechanisms via which psychosocial stress leads to asthma development.

Several cross-sectional studies have investigated cortisol levels in asthmatics and non-asthmatics, with diverse results. Not only lower [9-11] or normal cortisol levels [12-16] were reported in asthmatics compared to non-asthmatics, but also higher [17,18] cortisol levels. Since all studies investigated different aspects of the circadian pattern of cortisol secretion in relation to asthma, their results are incomparable.

Because psychosocial stress triggers asthma exacerbations [1], it could be argued that dysfunctions of the HPA-axis become more evident under stressful conditions. One previous study investigated both basal cortisol levels and cortisol responses to a laboratory stress task (Trier Social Stress Test) in asthmatics and non-asthmatics [12]. Although this study found no differences in basal cortisol levels between asthmatics and non-asthmatics, cortisol levels to a laboratory stress task were lower in asthmatics compared to non-asthmatics, indicating hyporesponsiveness of the HPA-axis which became only evident under exposure to stress.
Because of the small sample size of this study (asthmatics n=17, non-asthmatics n=18) these findings need to be replicated in a larger sample size.

In conclusion, results from previous cross-sectional studies have suggested that asthmatics have an altered HPA-axis function. However, it is unclear whether this alteration in HPA-axis precedes the development of asthma or is the result of asthma. To our knowledge, no previous longitudinal study is performed investigating alterations in HPA-axis function as an etiologic mechanism contributing to the development of asthma. We hypothesized that 1) there is a cross-sectional association between low cortisol and asthma, 2) low cortisol levels precede the development of asthma, and 3) adolescents with asthma have a blunted cortisol response upon exposure to stress.
Materials and methods

Study participants

The TRacking Adolescents’ Individual Lives Survey (TRAILS) is a prospective cohort study among Dutch adolescents. A detailed description of the sampling procedure and methods has been published previously [19]. Briefly, the TRAILS target sample involved 10–12-year-olds (born between 1 October 1989 and 30 September 1990 (first two municipalities) or between 1 October 1990 and 30 September 1991 (last three municipalities)) living in five municipalities in the North of the Netherlands, including both urban and rural areas. A total of 135 primary schools were invited to participate, encompassing 3483 eligible children. Of the 135 schools 13 refused to participate, resulting in the exclusion of 338 children. Of the 3145 remaining eligible children, 210 were excluded because they were either unable to participate or incapable to participate due to severe mental retardation or due to a serious physical illness or handicap, or if no Dutch-speaking parent or parent surrogate was available (Turkish and Moroccan parents who were unable to speak Dutch were interviewed in their own language). After intensive recruitment efforts (including telephone calls, reminder letters and home visits), a total of 2230 children (76.0%) were included in the study at baseline. So far, three assessment surveys have been completed (n=2230, mean age ± SD 11 ± 0.6 years; n=2149, 14 ± 0.5 years; n=1816, 16 ± 0.7 years).

Cortisol collection at age 11 years

Cortisol was assessed from saliva at three times during one day (shortly after waking up (Cort7.00AM), 30 minutes after waking up (Cort7.30AM) and at 8.00 PM (Cort8.00PM)) at age 11 years. Saliva samples were received from 1768 adolescents (details on collection and
assays in [20]). Non-responders did not differ from responders in terms of sex; non-responders were slightly older (mean age 11.2 vs. 11.1 year) [20].

**Stress test and cortisol collection at age 16 years**

At age 16 years, 715 adolescents performed a stress test, based on (but not identical to) the Trier Social Stress Task [21]. 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. The speech was followed by a 3-minute interlude in which the adolescents were not allowed to speak. In the second part, adolescents were asked to perform a 6-minute mental arithmetic task. The adolescents were instructed to repeatedly subtract the number 17 from a larger sum, starting with 13,287. The mental arithmetic task was followed by a 3-minute period of silence, after which the adolescents were debriefed about the experiment. Adolescents with a high risk of mental health problems were overrepresented in this population (details in the Online Depository).

Cortisol was assessed from saliva collected prior to the stress test (Cort1), directly after (Cort2), 20 minutes after (Cort3) and 40 minutes after the stress test (Cort4) (Details on collection and assays in [22]). Non-responders did not differ from responders in terms of sex; non-responders were slightly older (mean age 16.4 vs 16.1 year) [23].

**Asthma**

Data on asthma were collected via questionnaires at age 11, 14 and 16 years [24]. Asthma was defined as having a doctor diagnosis (Did a physician give your child a diagnosis of asthma?) (assessed at age 11 years) and/or symptoms and/or treatment for asthma in the past 12 months
Asthma development was defined as having asthma at a specific survey, while not having asthma at all previous surveys.

**Statistical analysis**

AUCg (11 years), AUCg (stress-induced), AUCi (11 years) and AUCi (stress-induced) were calculated [20,25,26] (formulas in the Online Depository). To correct for skewed distributions, cortisol values above or below 3 SD of the mean were regarded as outliers and excluded, after which all cortisol values were transformed to z-scores to normalize the data in order to be able to compare results on different cortisol measures. Including adolescents with cortisol values below or above 3 SD of the mean in the analysis did not changes the results. Logistic regression analyses were used to study associations between basal cortisol and asthma at age 11 years and between basal cortisol and asthma development at age 14 and 16 years. All analyses were adjusted for sex and quadratic effect of sampling month, since these are known to be associated with cortisol values [20] and/or asthma [24] in this cohort.

Furthermore, logistic regression analyses were used to study associations between cortisol response to stress test and asthma at age 16 years, adjusted for sex and sampling weights to correct for the oversampling on high risk of mental health problems, and in case of AUCi (stress-induced) also for baseline cortisol level (Cort1) (Online Depository).

All analyses were repeated adjusting for depression (affective problems scale of the Youth Self-Report [27]), physical activity and smoking in case of basal cortisol, and additionally adjusting for age, BMI and OC use in case of stress-induced cortisol. A previous study in this cohort showed that age and BMI were not related to basal cortisol [20].

To examine the impact of (inhaled and oral) steroid treatment on the above studied associations, sensitivity analyses were performed by excluding adolescents who used
corticosteroid medication at age 11 years, in case of basal cortisol, or at age 16 years, in case of stress-induced cortisol.

Statistical analyses were performed using SPSS Inc. Windows 18.0. P-values < 0.05 (tested 2-sided) were considered to be significant.
Results

Study population

Table 1 shows the characteristics of the study populations stratified according to asthma at age 11 years. Twenty-two (1%) adolescents used corticosteroid-containing medication. There was no significant difference in age, sex and cortisol levels between adolescents with and without asthma at age 11 years. However, adolescents with asthma used significantly more corticosteroid-containing medication compared to adolescents without asthma.

In 489 (30%) adolescents, the AUCi (11 years) was negative (cortisol levels measured at 7.00 AM were higher compared to cortisol levels measured at 7.30 AM). At age 11 years, no significant differences were found between adolescents with a positive or a negative AUCi with respect the proportion of adolescents with asthma: 34 out of 474 (7%) adolescents with a negative AUCi had asthma vs. 73 out of 1122 (7%) adolescents with a positive AUCi had asthma (Chi-square = 0.24, p=0.63).

Cross-sectional associations between cortisol levels at age 11 years and asthma

Logistic regression analyses, adjusted for sex and quadratic effect of sampling month, showed no significant cross-sectional associations between asthma at age 11 years and AUCg (11 years), AUCi (11 years) or Cort8.00PM (Table 2). Additionally adjusting for depression, physical exercise and smoking did not essentially affect the results. In addition, excluding adolescents using corticosteroid-containing medication did not essentially affect the results.
Longitudinal association between cortisol levels at age 11 years and asthma development at age 14 or 16 years

Logistic regression analyses, adjusted for sex and quadratic effect of sampling month, showed no significant association between AUCg (11 years), AUCi (11 years) or Cort8.00PM, and asthma development at age 14 years, or age 16 years (Table 2). Additionally adjusting for depression, physical exercise and smoking did not essentially affect the results. In addition, excluding adolescents using corticosteroid-containing medication did not essentially affect the results.

Study population stress-induced cortisol

Table 3 presents the characteristics of the study population participating in the stress experiments, stratified according to asthma at age 16 years. Thirty-four (5%) adolescents used corticosteroid-containing medication. There was no significant difference in age, sex and cortisol levels between adolescents with and without asthma at age 16 years. Adolescents with asthma used significantly more corticosteroid-containing medication compared to adolescents without asthma.

Cross-sectional association between cortisol levels during the stress test and asthma at age 16 years

Logistic regression analysis, adjusted for sex and sampling weights to correct for the oversampling on high risk of mental health problems, showed no significant association between AUCg (stress-induced) and asthma at age 16 years (OR (95%CI), 0.94 (0.67-1.31)). Comparable results were found for the association between AUCi (stress-induced) and asthma
at age 16 years (1.04 (0.74-1.47)). Additionally adjusting for age, depression, physical exercise, smoking, BMI and OC use did not essentially affect the results. In addition, excluding adolescents using corticosteroid-containing medication did not essentially affect the results.
Discussion

The present study did not show an association between basal or stress-induced cortisol levels and asthma when analyzing the data cross-sectionally. Furthermore, we found no association between basal cortisol levels and the development of asthma in the longitudinal analyses.

This is the first study that investigated associations between basal cortisol levels and asthma in a large study population (n=2230 adolescents). We found no association between basal cortisol measured at age 11 years and asthma at age 11 years. Previous studies showed lower [9-11], comparable [12-16] and higher [17,18] cortisol levels in asthmatics than non-asthmatics. We therefore have performed a post hoc meta-analysis to obtain a stronger conclusion about the association between cortisol and asthma. To allow pooling across studies that used different types of HPA axis measurements, we calculated a standardized mean difference (SDM (95%CI)) of basal cortisol levels in the morning and basal cortisol levels in the evening (see Online Depository for more details, Table E1 and E2) [28]. Both morning and evening cortisol levels were lower in asthmatics than non-asthmatics, however, the summary estimates were not significant (Figures 1 and 2). Funnel plots of the morning and evening cortisol levels indicated a possible publication bias (see Online Depository Figure E1 and E2), suggesting unidentified unpublished articles, which are mostly negative or neutral articles. This potential publication bias would be compatible with the fact that our study, despite having the largest sample size published, failed to find any significant association between cortisol and asthma or asthma development. It again underlines the importance of publishing studies that report the absence of a significant association, especially those with large sample sizes.
This study is the first one investigating the research question whether alterations in HPA-axis function precede asthma development. Neither asthma development at age 14 years, nor asthma development at age 16 years was significantly associated with basal cortisol levels upon awakening or evening cortisol level measured at age 11 years. Therefore, we did not find evidence that an alteration in the HPA-axis function precedes asthma development between ages 14 and 16 years. However, this study cannot rule out that alterations in HPA-axis function precede adult onset asthma.

Contrary to a previous study that showed a blunted cortisol response to stress in asthmatics [12], our study did not find such an association. Differences in study population and sample sizes could explain these inconsistent findings. The previous study included children (n=35, age 7-13 years), whereas our study included adolescents (n=715, age 16 years). Other explanations could involve differences in the time points at which cortisol was measured, and differences in the way the associations between cortisol levels to a stress task and asthma were studied. The previous study measured cortisol 35, 25, 15 and 1 minutes before and, 10, 20, 30 and 40 minutes after the stress test and compared individual time points between asthmatic and healthy children. Our study measured cortisol at the start, directly after, 20, and 40 minutes after the end of the stress test and investigated whether AUC was associated with asthma. When investigating individual cortisol levels measured at 20 and 40 minutes after the stress test in our study, we found no differences in cortisol levels between asthmatics and non-asthmatics (U = 12731.0, p=0.98 and U = 11453.0, p = 0.39 respectively). Therefore, our data suggest that cortisol responses upon exposure to stress are not associated with asthma.
The strength of this study is its longitudinal study design, allowing us to study the effect of cortisol prior to the onset of asthma. In addition, our study was performed in a large sample of the general population, thereby increasing the probability that our findings with respect to basal cortisol levels are generalizable to the population at large. However, this advantage of the large sample size comes with some associated disadvantages, especially the level of detail that was reached in the phenotyping of asthma and in the characterization of HPA-axis activity in our study. The asthma population in our study is heterogeneous in terms of phenotype, severity and management. Therefore, we may have missed potential associations with cortisol in specific subgroups such as those with nocturnal asthma or allergic asthma. We do not have information about whether adolescents with asthma have allergic or non-allergic asthma. However, we do have information about the presence of allergy, hay fever and eczema, which made it possible to give some indication whether asthmatic adolescents in our study have allergic (asthma with allergy, hay fever or eczema) or non-allergic asthma (asthma without allergy, hay fever and eczema). Sensitivity analysis revealed comparable associations with cortisol for allergic and non-allergic asthma (data not shown).

With respect to the characterization of the HPA-axis activity, cortisol was assessed at one day in our study. Previous studies showed that cortisol levels have notable intra-individual variability [12,16], which is reduced when sampling on a workday, as a result of the usually strict schemes [29]. In our study, cortisol was mostly assessed during school days, which are highly scheduled as well, so we expect that the same reduction in intra-individual variability applies to our adolescents. In addition, we expect that the large sample size will compensate for the possible reduction in reliability as a result of one day cortisol assessment, so random fluctuations in individual values will be set off.

Second, cortisol was assessed at three time points, namely shortly after waking up (still lying in the bed), 30 minutes after waking up and at 8.00 PM. As a consequence only the
cortisol awakening response and cortisol levels at 8.00 PM could be studied in relation to asthma and asthma development, and not the circadian rhythm of the cortisol secretion. Therefore, we cannot rule out that there is an association between specific elements of this circadian rhythm of cortisol secretion and asthma presence or development.

We were unable to find evidence suggesting a role for cortisol in asthma, despite its well-established anti-inflammatory properties and therapeutic effects when given as inhaled or oral corticosteroids. The question arises whether our study indicates a real lack of association, or whether it merely reflects the problems associated with large cohort studies. Further prospective studies are needed to unravel the role of cortisol in asthma or asthma development. It is pivotal for such studies that both cortisol and asthma are measured in detail. For cortisol, this implies measuring multiple time points and multiple days, in order to obtain a reliable and robust estimate of HPA-axis activity. For asthma, detailed phenotyping would enable to study differences in the association with cortisol in relation to asthma severity and medication use, and also in relation to subtypes including glucocorticoid resistant asthma or non-allergic asthma. Complaints of nocturnal worsening are especially interesting because of circadian rhythm of cortisol secretion and the anti-inflammatory effect of cortisol.

In summary, our study did not find evidence supporting a role for cortisol in asthma and asthma development. In addition, our study found no association between stress-induced cortisol levels and asthma. Further longitudinal studies from birth onward have to assess whether there exists a window of time in which alterations in HPA-axis function may contribute to asthma development. However, at adolescence, it seems unlikely that this plays a dominant role.
Acknowledgements:

This research was funded by a grant of the Netherlands Asthma Foundation (grant no 3.4.07.034). This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS). Participating centers of TRAILS include various departments of the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in the Netherlands. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 452-04-314 and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005; NWO Longitudinal Survey and Panel Funding 481-08-013), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), Biobanking and Biomolecular Resources Research Infrastructure BBMRI-NL (CP 32), and the participating universities. We are grateful to all adolescents, their parents and teachers who participated in this research and to everyone who worked on this project and made it possible.
References


Table 1: Characteristics of the study population stratified according to asthma at age 11 years

<table>
<thead>
<tr>
<th></th>
<th>Asthma (n=154 (8%))</th>
<th>No asthma (n=1893 (92%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years, mean ± SD</td>
<td>11.2 ± 0.6</td>
<td>11.1 ± 0.6</td>
</tr>
<tr>
<td>Females</td>
<td>77 (50%)</td>
<td>966 (51%)</td>
</tr>
<tr>
<td>Cort7.00AM nmol/l</td>
<td>11.7 (0.7-100)</td>
<td>10.9 (0.9-73.3)</td>
</tr>
<tr>
<td>Cort7.30AM nmol/l</td>
<td>14.4 (2.0-100.0)</td>
<td>14.9 (0.2-131.0)</td>
</tr>
<tr>
<td>Cort8.00PM nmol/l</td>
<td>1.5 (0.0-8.2)</td>
<td>1.7 (0.0-8.9)</td>
</tr>
<tr>
<td>AUCg (11 years)</td>
<td>6.6 (1.1-13.7)</td>
<td>6.6 (0.5-15.5)</td>
</tr>
<tr>
<td>AUCi (11 years)</td>
<td>0.8 (-3.2-4.8)</td>
<td>1.0 (-5.3-7.3)</td>
</tr>
<tr>
<td>Corticosteroid-containing medication</td>
<td>13 (11%)*</td>
<td>9 (1%)</td>
</tr>
</tbody>
</table>

Data are presented as median (range) or number and percentage of non-missing values between brackets unless stated otherwise; Cort7.00AM = cortisol measured shortly after waking up; Cort7.30AM = cortisol measured 30 minutes after waking up; Cort8.00PM = cortisol measured at 8.00 PM; AUCg = area under the curve with respect to the ground at age 11 years; AUCi = area under the curve with respect to the increase at age 11 years; * p < 0.05
Table 2: Estimated OR (95% CI) of the association between basal cortisol measured at age 11 years and asthma or asthma development adjusted for sex and quadratic effect of sampling month

<table>
<thead>
<tr>
<th></th>
<th>Asthma at age 11 years</th>
<th>Asthma development at age 14 years</th>
<th>Asthma development at age 16 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUCg (11 years)*</td>
<td>0.99 (0.80-1.23)</td>
<td>1.46 (0.97-2.19)</td>
<td>1.27 (0.76-2.12)</td>
</tr>
<tr>
<td>AUCi (11 years)*</td>
<td>0.91 (0.73-1.12)</td>
<td>1.14 (0.74-1.76)</td>
<td>1.06 (0.63-1.76)</td>
</tr>
<tr>
<td>Cort8.00PM*</td>
<td>0.89 (0.71-1.11)</td>
<td>1.26 (0.85-1.86)</td>
<td>1.12 (0.67-1.86)</td>
</tr>
</tbody>
</table>

* = z-scores; AUCg = area under the curve with respect to the ground at age 11 years; AUCi = area under the curve with respect to the increase at age 11 years; Cort8.00PM = cortisol measured at 8.00 PM at age 11 years; OR = odds ratio
Table 3: Characteristics of the population participating in the stress test according to asthma at age 16 years

<table>
<thead>
<tr>
<th></th>
<th>Asthma (n= 42 (6%))</th>
<th>No asthma (n=621 (94%))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age years, mean ± SD</td>
<td>16.1 ± 0.6</td>
<td>16.2 ± 0.6</td>
</tr>
<tr>
<td>Females</td>
<td>23 (55)</td>
<td>315 (51)</td>
</tr>
<tr>
<td>Cort1 nmol/l</td>
<td>3.0 (0.3-10.8)</td>
<td>3.2 (0.0-70.1)</td>
</tr>
<tr>
<td>Cort2 nmol/l</td>
<td>4.3 (0.5-9.6)</td>
<td>3.8 (0.0-68.0)</td>
</tr>
<tr>
<td>Cort3 nmol/l</td>
<td>3.7 (1.1-11.8)</td>
<td>3.7 (0.0-55.5)</td>
</tr>
<tr>
<td>Cort4 nmol/l</td>
<td>2.8 (0.9-16.4)</td>
<td>3.3 (0.0-51.5)</td>
</tr>
<tr>
<td>AUCg</td>
<td>226.3 (56.0-588.0)</td>
<td>234.0 (16.5-855.8)</td>
</tr>
<tr>
<td>AUCi</td>
<td>36.5 (-244.0-372.5)</td>
<td>16.5 (-359.5-448.3)</td>
</tr>
<tr>
<td>Corticosteroid-containing</td>
<td>22 (52)*</td>
<td>11 (2)</td>
</tr>
</tbody>
</table>

Data are presented as median (range) or number and percentage of non-missing values between brackets unless stated otherwise; Cort1 = cortisol measured just before the stress test; Cort2 = cortisol measured direct after the stress test; Cort3 = cortisol measured 20 min after the stress test; Cort4 = cortisol measured 40 min after the stress test; AUCg = area under the curve with respect to the ground (stress-induced); AUCi = area under the curve with respect to the increase (stress-induced); * p < 0.05
Figure 1: Forest plot of the standardized mean differences morning cortisol levels in asthmatics and non-asthmatics.

<table>
<thead>
<tr>
<th>Study Name</th>
<th>N</th>
<th>Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rakke (2011)</td>
<td>109</td>
<td>-0.486 (-0.879, -0.091)</td>
</tr>
<tr>
<td>Buske-Kirschbaum (2003)</td>
<td>35</td>
<td>0.033 (-0.630, 0.696)</td>
</tr>
<tr>
<td>Nomura (1997)</td>
<td>74</td>
<td>-0.454 (-0.916, 0.009)</td>
</tr>
<tr>
<td>Haen (1991)</td>
<td>18</td>
<td>0.543 (-0.405, 1.492)</td>
</tr>
<tr>
<td>Sutherland (2003)</td>
<td>31</td>
<td>1.257 (0.453, 2.060)</td>
</tr>
<tr>
<td>Oricac (1986)</td>
<td>28</td>
<td>-0.076 (-0.332, 0.779)</td>
</tr>
<tr>
<td>Landstra (2002)</td>
<td>46</td>
<td>-0.491 (-1.092, 0.110)</td>
</tr>
<tr>
<td>Kallenbach (1988)</td>
<td>24</td>
<td>-0.090 (-0.893, 0.713)</td>
</tr>
<tr>
<td>Overall</td>
<td></td>
<td>-0.446 (-1.114, 0.222)</td>
</tr>
</tbody>
</table>
N = study population; test for heterogeneity Q-test Chi-square = 70.04 df = 8, p<0.0001; I² = 0.89; df = degrees of freedom
Figure 2: Forest plot of the standardized mean differences evening cortisol levels in asthmatics and non-asthmatics.
N = study population; test for heterogeneity Q-test Chi-square = 7.70 df = 3 p = 0.05; I² = 0.61; df = degrees of freedom