

## Exposure to Aircraft Noise and Saliva Cortisol in Six European Countries

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No competing interests are declared.

**List of abbreviations and definitions used in the manuscript.**

BMI – Body Mass Index

CI – Confidence Interval

GIS – Geographical Information System

HPA - Hypothalamus-Pituitary-Adrenal

HYENA – HYpertension and Exposure to Noise near Airports

ICBEN – International Commission on Biological Effects of Noise

INM – Integrated Noise Model

UK – United Kingdom

WHO-World Health Organization

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## Abstract

### *Background*

Several studies show an association between exposure to aircraft or road traffic noise and cardiovascular effects, which may be mediated by a noise-induced release of stress hormones.

### *Objective*

Our objective was to assess saliva cortisol concentration in relation to exposure to aircraft noise

### *Method*

A multi-centre cross-sectional study, HYENA (Hypertension and Exposure to Noise near Airports) comprising 4861 persons was carried out in six European countries. In a subgroup of 439 study participants, selected to enhance the contrast in exposure to aircraft noise, saliva cortisol was assessed three times (morning, lunch and evening) during one day.

### *Results*

An elevation of 6.07 nmol/l (95% confidence interval (CI) = 2.32-9.81) in morning saliva cortisol level was observed in women exposed to aircraft noise above 60 dB( $L_{Aeq,24h}$ ) compared to women exposed to 50 dB( $L_{Aeq,24h}$ ) or lower, corresponding to an increase of 34%. Employment status appeared to modify the response. No association between noise exposure and saliva cortisol levels was found in men.

### *Conclusions*

Our results suggest that exposure to aircraft noise increases morning saliva cortisol levels in women, which could be of relevance for noise related cardiovascular effects.

## Introduction

Transportation noise is a significant and increasing problem in urban areas worldwide (WHO 2000). There is mounting evidence of an association between road traffic as well as aircraft noise and cardiovascular outcomes (Babisch et al. 2005; Babisch 2006; G Belojevic et al. 2008; de Kluizenaar et al. 2007; Eriksson et al. 2007; Leon Bluhm et al. 2007; Rosenlund et al. 2001; van Kempen et al. 2002; Willich et al. 2006) One proposed biological mechanism implies that noise causes a release of stress hormones, which in turn adversely affect cardiovascular risk factors (Babisch et al. 2001; Ising and Kruppa 2004; Spreng 2000). An intermediary mechanism may involve the metabolic syndrome in which a disturbed Hypothalamus-Pituitary-Adrenal (HPA) axis regulation has been assumed to play an important role (Chandola et al. 2006). The glucocorticoid hormone cortisol is the main secretory product of the neuroendocrine cascade and a valid indicator of stress (Evans et al. 2001; Miki et al. 1998; Schulz et al. 1998). The cortisol profile normally shows a diurnal variation, high in the morning and low at night (Hofman 2001). Following long time stressful exposure the ability to down-regulate cortisol may be inhibited (Spreng 2000).

Stress hormone studies on community noise exposure have generally been performed using urine and blood measurements (Babisch et al. 2001; Babisch 2003; Dallman 1993; Evans et al. 2001; Maschke 2003; Miki et al. 1998; Pruessner et al. 1999; Schulz et al. 1998). Saliva cortisol measurements are easy to perform and reliably reflect free cortisol levels in blood (Hofman 2001) and have recently been used in a few studies on exposure to road traffic and aircraft noise (Poll et al. 2001; Stansfeld et al. 2001; Waye et al. 2003). In two review articles, the relationship between road traffic noise as well as aircraft noise and cortisol was investigated (Babisch 2003; Ising and Kruppa 2004). Six out of the fourteen studies under

review showed an increase in cortisol level related to exposure, however, these were mainly based on urine measurements and with small sample sizes. Only two of the studies used saliva cortisol measures and the results were inconclusive (Poll et al. 2001; Stansfeld et al. 2001). Thus, the association between community noise exposure and cortisol levels is still unclear, particularly with regard to exposure-response relationships and gender differences.

In a multi-centre study (HYENA) including six European countries, there was an association between night-time aircraft noise as well as average daily road traffic noise exposure and risk of hypertension (Jarup et al. 2008). An acute blood pressure increase was also related to aircraft or road traffic noise in a subsample (Haralabidis et al. 2008). Our objective was to study saliva cortisol as a possible marker of noise-induced stress in a subgroup from the HYENA project.

## Material & method

### *Study subjects*

The HYENA-study was based in six countries and focused on seven airports: United Kingdom (Heathrow), Germany (Tegel), The Netherlands (Schiphol), Sweden (Arlanda and Bromma), Greece (Athens) and Italy (Malpensa). For the main study men and women 45-70 years of age living in selected areas surrounding the specific airport were invited. A total of 4861 subjects (2404 men and 2457 women) participated. The participation rate differed between countries, from about 30% in Germany, Italy and the UK, to 46% in the Netherlands, 56% in Greece and 78% in Sweden. A non-responder analysis showed no significant differences in occurrence of cardiovascular risk factors between participants and non-responders.(Jarup et al. 2008) Men and women who had lived at their address for less than 5 years or lived at their address less than 6 months a year were excluded from the main study as well as subjects who were too ill to participate or could not comprehend the questionnaire.

The sample for the saliva study was drawn from the HYENA main study. All participants were eligible for saliva sampling except shift workers, who were excluded since cortisol levels are related to the circadian rhythm and vary with the work schedule. To increase contrast in exposure participants with the highest and lowest levels of exposure to aircraft noise in each country were selected for the saliva sampling. A total of 84 subjects from each of the six participating countries were to provide saliva samples with the aim to recruit a total number of 500 participants. The participation rate was high in Sweden, UK and the Netherlands (99, 98 and 85%), lower in Italy (49%) and Germany (26%) and was not



recorded in Greece. A final sample of 439 participants (209 men and 230 women) participated (Table 1). The study has been approved by ethical committees in each of the participating countries and the participants gave their informed consent prior to the study.

Insert Table 1

#### *Assessment of noise exposure and risk factors*

The study subjects were interviewed at home by a nurse using a standardized questionnaire with focus on known risk factors for cardiovascular disease such as health status, socioeconomic and lifestyle factors (diet, physical activity, smoking habits). The participants also underwent blood pressure measurements and height as well as weight was assessed to calculate BMI (Body mass index). This procedure is described in detail elsewhere (Jarup et al. 2005)

Aircraft noise exposure was assessed for each participant's home address using noise maps from each of the participating countries (Jarup et al. 2005). Briefly, the home addresses were transformed into coordinates and marked on a GIS map (Geographical Information System) with contours in 1 dB intervals for aircraft noise. The dB-levels were calculated using three different periods of the day and aircraft noise data from the year 2002. This resulted in the following five variables: Leq24h (average sound level for 24 hours), Lden (average sound level for 24 hours, +5dB in evening, +10 dB at night), Ld (average during day), Le (average

during evening) and Ln (average during night). The noise maps were calculated with the Integrated Noise Model (INM) (Gulding et al. 1999) in all participating countries, except for the United Kingdom where the Ancon model was applied. (Ollerhead et al. 1999)

For road traffic noise local models were used. *The Good Practice Guide for Strategic Noise Mapping* (WG-AEN 2006) was used to merge the data between countries. In most countries only 24-h data on the intensity of road traffic were available. The variables Leq,24h and Ln<sub>night</sub> were both derived from these data and therefore no distinction could be made between the relative effects on cortisol level of road traffic noise exposure during the night or during the day. (Jarup et al. 2005)

### *Cortisol measurements*

The participants selected for saliva sampling received a kit with three test tubes, instructions, cover letter and return envelope. The subjects were instructed to collect the first sample half an hour after awakening (which usually corresponds to the peak excretion of cortisol), the second sample immediately before lunch and the third sample just before going to bed in the evening. Tooth brushing, smoking, food and drink intake were to be avoided thirty minutes before sampling. Detailed instructions were given to the participants to ensure that the samples were taken in a similar fashion for all participants in all countries and that the samples would be marked properly.

Each of the test tubes included a small cotton swab. The participants were instructed to put the swab in their mouth until it was completely soaked by saliva and then place it in the test tube, write the date and time on the label of the tube and put it in the refrigerator. After all samples were taken, the test tubes were either returned by post or collected by a fieldworker.

The samples were first centrifuged and frozen in a laboratory in each of the participating countries. When all samples had been received in each country, respectively, the saliva tubes were sent to a laboratory at Karolinska Institutet, Stockholm, Sweden, for analysis.

Cortisol levels in saliva were determined by the Spectria cortisol coated tube radioimmunoassay (RIA) kit, Orion Diagnostica, FI-02101 Espoo, Finland. All samples from each subject or group of subjects were analyzed simultaneously in duplicate. The within- and between assay coefficient of variation never exceeded 5.0 and 10.0%, respectively. A comparison of the cortisol analyses in 30 samples was made with the department of Physiological Psychology, University of Düsseldorf, Germany (Kirschbaum and Hellhammer 1999). There was a very high correlation (0.98) but a slight difference in level - with systematically lower levels in the Stockholm laboratory. The difference was 12.5% with 95% confidence limits 1.5-22.3%.

### *Statistical analyses*

Associations between noise exposure and saliva cortisol levels were analyzed using linear regression models including interaction terms between covariates. Results are mainly expressed as regression coefficients and 95% confidence intervals. A covariate was selected as a confounder if the inclusion of this variable in the preliminary model changed the

regression coefficient for noise exposure by more than 10%. A full model was then created and the covariates that affected the full model less than 3% were discarded in the final model.

The covariates included in the final fully adjusted regression model were aircraft noise (continuous), road traffic noise (continuous), country (six categories), age (continuous), gender (dichotomous), body mass index (continuous), alcohol use (continuous), diet (nine categories based on vegetable and fruit intake), employment status (three categories: employed, retired, other), occupational status (five categories: lower manual to higher managerial) noise reducing actions during night (dichotomous) and medication use (dichotomous). Four nighttime annoyance variables (railway, construction, industry and indoor) were categorized in accordance with the ICBEN eleven categorie scale) In the aircraft annoyance analyses the variable was classified in three categories (low=0-3, moderate=4-7, high=8-10). All information about potential confounders except for road traffic noise exposure was obtained from the questionnaire. Heterogeneity tests revealed no statistically significant differences between countries and fixed effect model were used in the combined analyses

The statistical analyses were performed with STATA 8.0 (Stata Corp., College Station, TX).

## Results

The cortisol levels in the morning sample showed a roughly normal distribution (figure 1).

Two “outliers” were observed in the morning samples, one in the United Kingdom (99.8) and one in Sweden (89.0). However, these values were still within the normal range and were therefore included in the subsequent analyses.

Insert figure 1

The median levels for morning, lunch and evening samples were comparable between countries (figure 2) with the exception of Greece, having a lower median level of the morning sample.

Insert figure 2

Table 2 shows linear regression coefficients and 95% CI for cortisol level in saliva from the morning sample in relation to aircraft noise levels  $\text{dB}(L_{\text{Aeq}24\text{h}})$ . For women a significant association was seen between aircraft noise exposure and morning saliva cortisol levels in the analysis based on a continuous exposure variable. This corresponds to a 5% increase in mean

cortisol level for each 5 dBA rise in exposure. Women with an aircraft noise level above 60 dBA had a significantly higher morning saliva cortisol concentration than women with an aircraft noise exposure below 50 dBA, the difference in means being 6.07 (2.32, 9.81) nmol/l, corresponding to a 34% increase. For men no association between aircraft noise and cortisol levels was seen. Separate analyses without the previously mentioned outliers (see figure 1) did not change the results.

Insert table 2

In country specific analyses, a statistically significant increase in morning cortisol levels in women exposed to air traffic noise was seen only for the United Kingdom (Heathrow), but there was no heterogeneity in results between the countries (table 3). No clear effects were indicated among men. It should be noted that the number of investigated subjects for either sex in each country was small, leading to substantial statistical uncertainty of the estimates.

Insert table 3

Women exposed to aircraft noise over 60 dBA had an increase in morning saliva cortisol level regardless of whether they considered themselves as annoyed or not. There was a 25% increase (4.86 nmol/l CI95% -0.46,10.18) in mean saliva cortisol for those who reported low annoyance, 52% increase (9.96 nmol/l 95%CI 3.30,16.62) nmol/l for moderately annoyed participants and a 30% increase (5.73 CI 95% 0.05, 11.40) for highly annoyed study subjects compared to participants with an exposure below 50 dBA and who reported low annoyance. There was no rise in saliva cortisol level among participants who reported moderate or high annoyance in the lowest exposure group <50dBA.

Employed women had higher morning saliva cortisol levels than retired women, particularly among those with high exposure to aircraft noise (table 4). Women exposed to aircraft noise above 60 dBA and who were currently employed had a 83% higher mean morning saliva cortisol level (16.23 (9.29, 23.2) nmol/l) compared with retired women exposed to aircraft noise level at 50dBA or lower. Retired women exposed to aircraft noise above 60 dBA had an increase of 27% (5.38 (-0.57,11.33) nmol/l). Additional analysis restricted to participants 55 years of age or older showed a similar increase, indicating that the effect is not explained by age differences.

Insert table 4

The relation between aircraft noise and cortisol did not change noticeably using the five different aircraft noise variables. In principle, night flights may be of great importance for the morning saliva levels. However, morning flights might also affect the morning saliva levels but are not included in the  $L_{night}$  estimates. Limited night flights occurred for Berlin – Tegel (no flights between 22:00 and 05:00) and no night flights occurred for Stockholm – Bromma. Thus, imputed values were used for  $L_{night}$  estimates, which may contribute to a greater uncertainty of the estimates. Analyses were also performed using all saliva samples, including differences between day or evening and morning levels, but no consistently different associations were seen compared to those presented.

No apparent associations were found between road traffic exposure and saliva cortisol levels.

## Discussion

The main finding in this study was a significant exposure-response increase in cortisol levels in the morning sample for women exposed to aircraft noise. In the HYENA main study there was a significant exposure-response relationship between night-time aircraft noise exposure and risk of hypertension. (Jarup et al. 2008) This is in line with our findings in women, however we did not see any comparable association in men. The differences in noise influence on saliva cortisol between men and women are difficult to explain. However, it is of interest that recent studies have shown a relation between occupational stress and elevated morning saliva cortisol concentration only in women. (Alderling et al. 2006; Lundberg 2005; Maina et al. 2009; Rystedt et al. 2008) Previous studies regarding traffic noise exposure in relation to hypertension or myocardial infarction have been inconclusive regarding gender differences. (Babisch et al. 2005; GA Belojevic et al. 2008; Jarup et al. 2008; Selander et al. 2009; Willich et al. 2006)

We did not find any consistent effect of road traffic noise on morning saliva cortisol levels though there was a significant association between average daily road traffic exposure and hypertension in the HYENA study, primarily among men (Jarup et al. 2008). One explanation could be that the noise exposure contrast in our study was less for road traffic noise than for aircraft noise as a result of our selection focusing on subjects with high and low exposure to aircraft noise. As we only investigated a subgroup from the main study, this leads to less powerful analyses, which may contribute to the apparent lack of association.

The focus in this study was not on pathological or abnormal saliva cortisol levels but rather on differences in distribution of values in different exposure groups. In individual cases at least five samples during the day and at least three sampling days would be needed for a reliable individual assessment of saliva cortisol. On a group level, however, three samples per person



during one sampling day may be sufficient (Bigert et al. 2005). The first rise in the morning 30 minutes from awakening has been pointed out as being particularly relevant as it may reflect long lasting stress levels (Kirschbaum and Hellhammer 1999; Kudielka and Kirschbaum 2003).

The country specific analyses suggested a stronger association between morning cortisol levels and exposure to aircraft noise in the United Kingdom (Heathrow) although no statistically significant heterogeneity was indicated between the countries. London – Heathrow is a major airport with night flights and residents living close to the runways, while in the other countries restrictions in night traffic, smaller airports and/or rural residential areas next to the airport contribute to lower exposure levels. Among the participants in Greece the mean level of cortisol in the morning sample was lower than in the other countries. One reason for this could be that as the Greece airport (Athens) is new and the participants had been exposed for a much shorter time than the participants in the other countries. It is biologically plausible that a longer exposure time is needed to develop chronic stress reaction with increase in salivary cortisol levels. The response rates for the saliva sampling differed between countries. In Germany, Greece and Italy the response rate was low or not recorded. This can contribute to a lower validity, but it is uncertain how this affected the results.

In our study annoyance to aircraft noise did not seem to have a relation to morning saliva cortisol level. A rise in saliva cortisol was noted with increasing noise levels regardless of the annoyance level and in the highest exposure group (>60 dBA) there was an increase in saliva cortisol even among participants with low annoyance. In contrast, participants in the lowest exposure group did not show any change in cortisol level even if they were highly annoyed.

These findings suggest that the effect is not dependent on the subjective annoyance experience but rather connected directly to noise exposure.

It is also of interest that women who were employed had a higher cortisol level than retired women. A particularly strong increase was found in employed women exposed to high levels of aircraft noise. This effect could be a result of disrupted sleep during night and the lack of recovering during the day due to employment. It can also be a result of stressful activities related to employment (Alderling et al. 2006; Lundberg 2005; Maina et al. 2009; Rystedt et al. 2008) in combination with aircraft noise exposure at home. Further variables that may influence the results are marital status and number of children. Unfortunately, we lacked information regarding these factors, but adjusting for number of occupants in the participant's household did not change the estimates markedly.

In conclusion, there was a significant increase in cortisol levels in the morning for women exposed to aircraft noise. No comparable association was found in men. Our results provide some support for a physiological stress reaction induced by noise which may contribute to hypertension and other adverse cardiovascular effects.

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Table 1. Number of participants in the saliva sampling part of the HYENA study by country and noise exposure at residence.

Country	Aircraft noise exposure ( $L_{Aeq, 24h}$ )			Subjects per country
	<50	≥50 -<60	≥60	
United Kingdom	35	5	47	87
Germany	43	17	19	79
Greece	10	48	10	68
The Netherlands	23	16	23	62
Italy	19	27	12	58
Sweden	44	29	12	85
Subjects per noise category	174	142	123	439

Table 2. Linear regression coefficients for the relation between air traffic noise exposure and morning saliva cortisol levels among 439 subjects in 6 European countries.<sup>a</sup>

dB L <sub>Aeq,24h</sub>	All		Women		Men	
	n	Coefficient (CI 95%)	n	Coefficient (CI 95%)	n	Coefficient (CI 95%)
Continuous Per 5 <sup>b</sup>	439	0.25 (-0.17,0.66)	230	0.80 (0.26,1.34)	209	-0.33 (-0.88,0.22)
Categorical <50 <sup>c</sup>	174	-	97	-	77	-
≥50-<60	142	1.04 (-1.61,3.68)	77	2.16 (-1.26, 5.59)	65	0.06 (-3.64,3.76)
≥60	123	1.83 (-0.90,4.35)	56	6.07 (2.32, 9.81)	67	-2.00 (-5.61,1.61)

<sup>a</sup> All analyses adjusted for road traffic, country, age, gender (only for “All”), employment status, occupational status, medication use, BMI, alcohol, diet, remedy during night, work noise and other noise sources in living environment.

<sup>b</sup> Rise in cortisol in nmol/l per 5dB increase in noise level.

<sup>c</sup> Reference category, arithmetic mean cortisol level: all = 19.13, women = 17.7, men = 20.92.

Table 3. Linear regression coefficients for the relation between air traffic noise exposure and morning saliva cortisol levels among 439 subjects in 6 European countries. <sup>a</sup>

Country	n	Women	n	Men
		Coef per 5 dBA (95% CI) <sup>b</sup>		Coef per 5 dBA (95% CI) <sup>b</sup>
The United Kingdom	40	2.23 (0.45, 4.01)	47	0.52 (-1.00, 1.99)
Germany	43	0.02 (-1.73, 1.77)	36	0.11 (-1.74, 1.97)
The Netherlands	29	2.34 (-1.25, 5.93)	33	0.84 (-2.56, 4.25)
Sweden	46	1.09 (-0.12, 2.31)	39	0.05 (-1.25, 1.34)
Italy	43	-0.08 (-2.95, 2.79)	25	-0.85 (-4.06, 2.36)
Greece	29	-0.36 (-2.35, 1.64)	29	-1.10 (-3.30, 1.10)

<sup>a</sup> All analyses adjusted for road traffic, country, age, gender (only for “All”), employment status, occupational status, medication use, BMI, alcohol, diet, remedy during night, work noise and other noise sources in living environment

<sup>b</sup> Rise in cortisol in nmol/l per 5dB increase in noise level.



Table 4. Linear regression coefficients for the relation between air traffic noise exposure and morning saliva cortisol levels with regard to employment status among 230 women in 6 European countries. <sup>a</sup>

Employ. Status <sup>b</sup>	<50		Aircraft noise dBA		>60	
	n	Coef <sup>c</sup> (CI 95%)	n	50-60 Coef <sup>c</sup> (CI 95%)	n	Coef <sup>c</sup> (CI 95%)
Retired	33	-	24	0.62 (-5.18, 6.41)	24	5.38 (-0.57,11.33)
Other	17	1.62 (-4.94, 8.18)	25	5.36 (-0.91, 11.63)	15	3.90 (-3.27,11.07)
Employed	47	3.93 (-1.64, 9.49)	28	7.87 (1.63, 14.11)	17	16.23 (9.29,23.2)

a All analyses adjusted for road traffic, country, age, gender (only for “All”), occupational status, medication use, BMI, alcohol, diet, remedy during night, work noise and other noise sources in living environment.

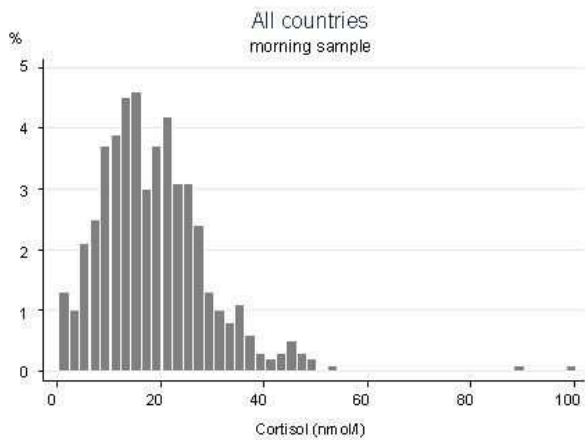
b Employment status is classified categorically. “Retired” includes retired participants. “Other” includes participants on sick leave, unemployed subjects, housewives and students etc. “Employed” included both full-time and part-time employment as well as self-employed working from home.

c Linear regression coefficients for morning saliva cortisol level in nmol/l  
Arithmetic mean cortisol level in the reference category=19.67 nmol/l

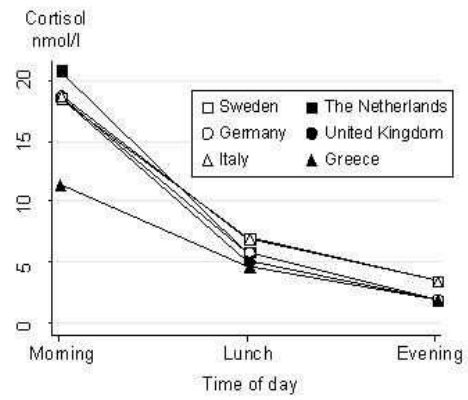
## Figure legends

Figure 1. Distribution of cortisol for morning saliva samples from 439 participants exposed to aircraft noise in six European countries.

Figure 2. Median cortisol level in nmol/l for each country for morning, lunch and evening saliva samples.



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30x22mm (600 x 600 DPI)