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Title: Effects of aircraft noise exposure on saliva cortisol near airports in France

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Anne-Sophie Evrard (ASE) and Bernard Laumon (BL) with Jacques Lambert (JL) and Patricia Champelovier (PC) conceived and designed the study. ASE and Marie Lefèvre (ML) conducted the study. JL interpreted the aircraft noise data and PC interpreted the annoyance data. Marie-Christine Carlier (MCC) analyzed saliva samples and determined cortisol levels. ML was involved in data extraction and preparation and carried out the statistical analyses, supervised by ASE and BL. The analyses were interpreted by ASE, ML and MCC with BL, JL and PC. ML and ASE drafted the initial report; all coauthors revised the report and approved the final version. ASE is responsible for the overall content as the guarantor of this paper.

Abstract

Background: Saliva cortisol is a possible marker of noise-induced stress and could then mediate the relation observed between exposure to aircraft or road traffic noise and cardiovascular diseases. However, the association between transportation noise and cortisol levels is still unclear.

Objectives: The objective of the study was to investigate the variability of saliva cortisol concentration as an indicator of disturbed HPA axis regulation in relation to long-term aircraft noise exposure.

Methods: Saliva samples were taken when awakening and before going to bed for 1244 participants older than 18 years of age. Information about health, socioeconomic and lifestyle factors was also collected by means of a face-to-face questionnaire performed at home by an interviewer. Aircraft noise exposure was assessed for each participant's home address using noise maps. Linear regression models were used to evaluate the effects of aircraft noise exposure on the morning and evening cortisol levels and on the daily variation of cortisol per hour.

Results: This study suggests a modification of the cortisol circadian rhythm in relation to aircraft noise exposure. This exposure was associated with a smaller variation of cortisol levels over the day, with unchanged morning cortisol levels, but higher cortisol levels in the evening.

Conclusions: These findings provide some support for a psychological stress induced by aircraft noise exposure, resulting in HPA dysregulation and a flattened cortisol rhythm, thus contributing to cardiovascular diseases.

Key words: Epidemiology; aircraft noise exposure; saliva cortisol; HPA dysregulation

What this paper adds

- Although they were widely discussed, the effects of a long-term noise exposure including occupational, road, railway and air traffic noise on cortisol secretion are still unclear. The DEBATS study is the first to investigate the relationship between this exposure and saliva cortisol near French airports.

- The findings of the present study suggests a modification of the cortisol circadian rhythm in relation to aircraft noise exposure. This exposure was associated with a smaller variation of cortisol levels over the day, with unchanged morning cortisol levels, but higher cortisol levels in the evening.

- Alteration in the cortisol rhythm has been associated with various negative outcomes like cardiovascular diseases. However, since the study of cortisol in large populations is a relatively new issue, epidemiological evidence contributing to explain the whole mechanism is still lacking. Future studies should necessarily pay more attention to the interaction with other stressors.

Introduction

Activation of the hypothalamus-pituitary-adrenal (HPA) axis and the subsequent release of cortisol are considered to be one of the major components of the physiological stress response in humans. Thus cortisol can be viewed as a reliable stress indicator.[1] Under natural unstimulated conditions, the secretion of cortisol follows a circadian rhythm characterized by a peak in the early morning hours, followed by declining cortisol concentrations throughout the day, reaching the lowest levels during the late evening.[2] In most healthy people, morning awakening is associated with a secretory episode, called the cortisol awakening response (CAR), and defined as the increase of cortisol levels within 20-30 minutes after awakening. The CAR is considered as a reliable measure for the acute activity of the HPA axis.[3] The circadian rhythm of cortisol is modified by altered sleep patterns and exposure to daily life and psychological stressors of many types.[4-9] Cortisol plays a crucial role in many metabolic and homeostatic processes in response to stress including, but not limited to, the activity of the heart, blood pressure, blood lipids and glucose, blood clotting and blood viscosity.[10] Modifications of these physiological markers are established risk factors for cardiovascular disease including hypertension and myocardial infarction.[11] The evidence of an association between noise exposure and cardiovascular outcomes has increased.[12-25] Noise could be considered as a psychosocial stressor that activates the sympathetic and neuroendocrine system, thus leading to a long-lasting activation of the HPA axis, and then to cardiovascular disease.

Although they were widely discussed, the effects of a long-term noise exposure including occupational, road, railway and air traffic noise on cortisol secretion are still unclear.[26] In fact most studies measured cortisol in urine, had small sample sizes and included children. The largest study to date, including 439 participants, the HYENA (HYpertension and Exposure to

Noise near Airports) study, suggests that exposure to aircraft noise increases morning saliva cortisol levels in women, but no similar association was found in men.[27] This study has been performed using saliva cortisol measurements: they are non-invasive in comparison with urine or blood measurements and salivary cortisol level can be considered as a reliable indicator of the plasma-free cortisol concentration.[28] The majority of the studies have focused on average levels of cortisol at specific times of the day[27,29] or on the CAR,[30] even if recent investigations have demonstrated that the CAR is different from the daily cortisol profile and can be seen as an additional phenomenon reflecting specific processes associated with awakening.[31]

With repeated stress exposures, the HPA axis becomes less flexible and there would be fewer differences between morning and evening cortisol levels.[10] Several studies showed an association between long-term stress exposure and a flattening of the diurnal cortisol rhythm across the day.[4-7,32-34] The variability of cortisol during the day could therefore be used as an indicator of a disturbed HPA axis regulation.

The DEBATS study (Discussion on the health effects of aircraft noise) is the first to investigate the relationships between long-term aircraft noise exposure and the health status of the French population living in the vicinity of airports. Based on data collected in 2013 at the participants' inclusion in this study, the present paper addresses more specifically the issue of an association between aircraft noise exposure and saliva cortisol. Not only morning or evening cortisol levels were analyzed, but also the variability of saliva cortisol during the day, as an indicator of disturbed HPA axis regulation in relation to aircraft noise exposure.

Methods

Study population

The DEBATS study population included persons older than 18 years of age at the time of the first interview, living near one of the three following French international airports: Paris-Charles de Gaulle, Toulouse-Blagnac, and Lyon Saint-Exupéry. Finally 1,244 participants (549 men and 695 women) were initially included in the main study.[12]

The sample for the saliva study was drawn from the DEBATS main study. All participants were eligible for saliva sampling. 74 shift workers and 82 subjects with atypical sleeping patterns were excluded from the analyses since disruption of circadian rhythms, especially due to working at night or to irregular periods of sleep, can lead to physiological disorders that alter the endocrine system. In addition, 55 participants with at least one missing saliva sample or with missing sampling time or date were excluded as well as the 75 participants with two requested saliva samples separated by 24 hours or more. Finally, 4 subjects whose medication (essentially treatments with corticosteroids) could interfere with the level of saliva cortisol were also removed from the analyses. A final sample of 954 participants (400 men and 554 women) was eligible for the cortisol variation analyses.

Questionnaire

For their inclusion in the study in 2013, the participants filled out a questionnaire during a face-to-face interview at their place of residence. Information was collected by an interviewer on demographic variables, socioeconomic status, lifestyle factors including smoking and alcohol consumptions and physical activity, personal medical history in terms of sleep disturbances, cardiovascular diseases, anxiety, depressive disorders, medication use, and finally annoyance due to the noise exposure. The participants also underwent blood pressure

and anthropometric measurements (weight, height and waist circumference). This procedure is described in detail elsewhere.[12]

Cortisol measurements

At the end of the questionnaire, interviewers gave the participants a kit with two test tubes (Starstedt, Nümbrecht, Germany) and instructions to collect saliva to ensure that the sampling procedure was correct and similar for all the participants. The subjects were requested to collect two saliva samples: the first one immediately after awakening (when the cortisol level is usually high) and the second one just before going to bed (when the cortisol level is usually the lowest). Wake-up and bedtime samples allowed us to study the individual slope of the cortisol variability during the day. The participants had to write the date and time of sampling on the label of each tube. Tooth brushing, smoking, food and drink intake were to be avoided 30 minutes before sampling. Each tube included a small swab that the participants had to put in their mouth and to chew for about one minute. The saliva samples had then to be stored in the fridge until the interviewer collected them and sent them to a laboratory in Lyon (France) where they were frozen in order to eliminate mucins before analysis. Cortisol levels in saliva were determined by the cortisol saliva ELISA (enzyme-linked immunosorbent assay) kit (IBL international, Hamburg, Germany). All samples from each subject were analyzed simultaneously in duplicate.

Aircraft noise exposure assessment

Aircraft noise exposure was assessed for each participant's home address using outdoor noise exposure maps produced by the French Civil Aviation Authority for Toulouse-Blagnac and Lyon Saint-Exupéry airports, and by Paris Airports. These maps output noise contours in 1-dBA intervals for each area. The home addresses of the participants were linked to these

contours using a geographic information system (GIS) technique. The dBA levels were calculated using three different periods of the day, resulting in the use of four noise indicators in the statistical analyses: the weighted average of sound levels from day (06:00 to 18:00), evening (18:00 to 22:00) and night (22:00 to 06:00), where evening and night sound pressure levels receive a 5 dB(A) and a 10 dB(A) penalty respectively (L_{den}), the average sound level for 24 hr ($L_{Aeq,24h}$), the average during the day and the evening ($L_{Aeq,16hr}$) and finally the average during the night (L_{night}). They were estimated with a 1-dBA resolution from a minimum of $L_{Aeq,24h}$ 45 dBA, L_{den} 45 dBA, $L_{Aeq,16hr}$ 35 dBA and L_{night} 30 dBA. Aircraft noise levels below these values were assigned 44 dBA for $L_{Aeq,24h}$ and L_{den}, 34 dBA for $L_{Aeq,16hr}$ and 29 dBA for L_{night}. The L_{den} indicator was used to select the participants (Table 1).

Statistical Analysis

The association between cortisol variation per hour and aircraft noise exposure was analysed. Cortisol variation was defined as the absolute difference between morning and evening saliva cortisol. Except the CAR, the classic diurnal pattern of cortisol is marked by a gradual drop throughout the rest of the waking hours.[35] The time interval between the two measurements varied between participants, even after exclusion of subjects with atypical sleeping patterns. Therefore, in order to enable comparison between individuals, cortisol variation per hour was calculated for each participant by dividing the cortisol variation by the number of hours between the two saliva samples. The distributions of cortisol levels were roughly right skewed even if it was less marked for morning saliva samples (Figures 1a and 1b). Therefore, linear regression models with log-transformed cortisol variation per hour as the outcome variable, and aircraft noise exposure and confounders as covariates were used to assess the association of aircraft noise exposure with saliva cortisol.

The first model (M0) included only aircraft noise exposure as an explanatory variable. The major potential confounders according to the literature were then added in the adjusted regression model (M1): the day of the week of the saliva sampling (weekday/weekend), age (continuous), gender (dichotomous), alcohol consumption (four categories: non/light/moderate/heavy drinker), smoking habits (four categories: non/ex/occasional/daily smoker), household monthly income (three categories: < 2300; 2300-4000; >=4000 euro), regular physical activity (dichotomous) and body mass index (BMI , body weight divided by height squared in continuous). As sleep disturbances, psychiatric disorders and annoyance may be intermediate steps in the causal chain between aircraft noise exposure and the cortisol secretion, sleep duration (five categories: ≤5h; 6h; 7h; 8h; ≥ 9h), psychiatric distress using the 12-item General Health Questionnaire (GHQ) with Likert-type scoring method (three categories: no apparent disorder; moderate disorders; severe disorders) and annoyance from aircraft noise exposure (two categories: annoyed/not annoyed) were added to the final fully adjusted regression model (M2). As aircraft noise levels were strongly correlated with the airports, the 'airport' variable was not included in the final model in order to avoid over-adjustment when aircraft noise levels and the area of study were both introduced in the multivariate model. To assess whether the country of birth (used as a proxy for ethnicity), marital status, the number of occupants in the participants' household, occupational activity, work-related stress and major life events, and the season of the saliva sampling would confound the effects of noise on cortisol, these variables were initially included in the multivariate regression models. However, they did not contribute significantly to the model and did not have any impact on the effect estimate of noise, so they were not included in the final model.

Linear regression models with log-transformed morning or evening cortisol levels as the outcome variable were used to assess the associations between aircraft noise exposure and morning and evening cortisol levels, at awakening and before going to bed separately. These models were adjusted on the same confounders as those included in the models with cortisol variation, in addition to the sampling time.

Linearity of the relation between the dependent variable and aircraft noise exposure was tested using generalized additive models including a smooth cubic function with linear and quadratic terms for aircraft noise exposure.[36] As the quadratic term was not significant in these models, associations with the continuous exposure variable per 10 dBA increase were finally estimated and presented in the present paper.

The use of log-transformed outcomes in linear regression models allows coefficients to be interpreted as the percentage change in outcome per unit change in the independent variable after applying the transformation: $\beta_{\%}$= [exp(coefficient) − 1] *100. To be able to easily interpret the results presented in Tables 3 and 4, an exponentiation was applied on the log-transformed estimates. Therefore, these results can be directly interpreted as relative risks.

Statistical analyses were conducted using SAS version 9.4 (SAS Software [program] 9.4 version. USA: Cary North Carolina, USA 2011).

Results

The average sampling time was very similar across aircraft noise exposure levels (L_{den}): 07.28 for the morning samples and 22.46 for the evening ones (Table 2). The average cortisol variation per hour was 2.2 nmol/L (standard deviation = 1.5). It was significantly lower for participants with aircraft noise levels higher than 60 dBA (L_{den}) compared to those with aircraft noise exposure lower than 55 dBA (L_{den}; p=0.003). Cortisol levels for the evening samples were significantly higher for participants with aircraft noise levels higher than 60 dBA compared to those with aircraft noise exposure lower than 55 dBA (p=0.0002). Cortisol levels for the morning samples were similar across noise exposure categories (p=0.26).

Table 3 shows linear regression coefficients after exponentiation and 95% CIs for cortisol variation in saliva per hour in relation to the major potential confounders. Cortisol variation was significantly associated with the day of the week of the saliva sampling (exp(β) = 1.47; 95%CI = 1.19-1.81 for a weekday compared to the weekend), a regular physical activity (exp(β) = 1.32; 95%CI = 1.13-1.53) and with household monthly income (exp(β) = 0.79; 95%CI = 0.66-0.95 for people with less than 2,300 euros (2,600 US$) per month compared to those with more than 4,000 (4,500 US$)). If additional factors that may be intermediate steps in the causal chain between aircraft noise exposure and cortisol secretion were included in the model, cortisol variation was also significantly associated with sleep duration. Individuals sleeping at least 9 hours had lower cortisol variations compared to those sleeping 7 hours per night (exp(β) = 0.81; 95%CI = 0.66-0.99). No significant effects were found for the other variables.

Linear regression coefficients after exponentiation and 95% CIs for cortisol variation per hour as well as morning and evening saliva cortisol levels in relation to aircraft noise exposure are presented in Table 4. Analyses were performed for each noise indicator (L_{den}, $L_{Aeq,24h}$, $L_{Aeq,16hr}$

and L_{night}) separately. A significant association between cortisol variation per hour and aircraft noise exposure was found whatever the noise indicator and whatever the model (M0, M1 or M2): participants with higher noise levels had a significantly lower cortisol variation compared to those with lower noise levels. The coefficients were very similar for the noise indicators including day-evening exposure (for M2: exp(β) = 0.85, 95%CI = 0.75-0.96 per 10 dBA increase in noise levels for L_{den}; exp(β) = 0.86, 95%CI = 0.74-0.99 for $L_{Aeq,24h}$; and exp(β) = 0.87, 95%CI = 0.77-0.99 for $L_{Aeq,16hr}$). The relation was even more significant when considering only night-time noise exposure (L_{night}, exp(β) = 0.83; 95%CI = 0.74-0.93). This corresponds to a decrease from 13% to 15% in cortisol variation per hour for each 10 dBA rise in day-evening-night (L_{den} ,$L_{Aeq,24h}$) and day-evening ($L_{Aeq,16hr}$) exposure. The decrease reached 17% for each 10 dBA rise in night-time exposure (L_{night}).

Aircraft noise exposure had no impact on the morning cortisol levels. For cortisol levels at night just before going to bed, a significant association between aircraft noise exposure and evening saliva cortisol levels was found, whatever the noise indicator and whatever the model (for M2: exp(β) = 1.17 per 10 dBA increase in noise levels for L_{den} [95%CI = 1.07-1.28], $L_{Aeq,24h}$ [95%CI = 1.06-1.29], L_{night} [95%CI = 1.08-1.27]; exp(β) = 1.15 per 10 dBA increase in noise levels for $L_{Aeq,16h}$ [95%CI = 1.05-1.25]). This corresponds to an increase from 15% to 17% in mean cortisol level for each 10-dBA rise in noise exposure.

The association was even stronger and more significant when the analysis was restricted to the 760 participants who had resided at their address for at least 5 years (for M2: exp(β) = 0.82 80 for day-evening-night and night noise indicators and exp(β)= 0.84 for $L_{Aeq,16hr}$).

The association between cortisol variation and aircraft noise exposure was significant in women, not in men but was of the same order of magnitude: exp(β) varied between 0.81 and 0.85 among women and between 0.88 and 0.91 among men. The relationship between aircraft

noise exposure and morning cortisol levels was not significant, neither in men nor in women, but a significant association was found with evening cortisol levels in both gender ($\exp(\beta)$ varied between 1.11 to 1.13 in women and between 1.15 to 1.19 in men). Moreover, when included in the models M0, M1 or M2, the interaction term between gender and noise was not significant ($p>0.50$).

Discussion

This study suggested a modification of the cortisol circadian rhythm in relation to aircraft noise exposure. This exposure was associated with a smaller variation of cortisol levels over the day, with unchanged morning cortisol levels but higher cortisol levels in the evening.

The DEBATS main study found a significant association between night-time aircraft noise exposure and the risk of hypertension in men only.[12] In this study, comparisons between male and female to ascertain whether sex steroids or menopausal status would influence HPA axis, showed no gender differences for the association between cortisol levels or variation and noise exposure.

The effects of a long-term noise exposure including occupational, road, railway and air traffic noise on cortisol secretion were largely discussed but are still unclear.[26] In fact most studies measured cortisol in urine, had small sample sizes and included children. Most previous studies regarding cortisol levels in relation to chronic stressors have been inconclusive about gender differences.[37-39] The largest study to date, the HYENA study included 439 participants, and suggests that exposure to aircraft noise increases morning saliva cortisol levels in women.[27] Unlike the results of the HYENA study, the ones observed in the present study provide some support to the hypothesis that with repeated stress exposures, the HPA axis becomes less flexible and there would be fewer differences between morning and evening cortisol levels.[10] The question to know whether high cortisol levels or disturbed regulation is due to aircraft noise exposure or to other adverse life conditions or individual physiological characteristics is of major importance. In this study, the individuals samples based on aircraft noise exposure were expected to be comparable with regard to life adversities and individual differences. Moreover, controlling for major confounders previously found in the literature (the day of the week of the saliva sampling, age, gender, alcohol consumption, smoking

habits, household monthly income, regular physical activity and body mass index)[40] did not change the results. In this study, the assessment of extensive covariate data made it possible to evaluate a large number of possible confounding factors and ensure the stability of the results. However, uncontrolled or residual confounding, exposure misclassification, and selection bias all need to be considered.

Crude estimates of the effects of aircraft noise exposure on cortisol levels or cortisol variation were very similar to the estimates adjusted on the established confounders and additional factors that may be intermediate steps between aircraft noise exposure and the cortisol secretion such as sleep duration, psychiatric distress and annoyance from aircraft noise exposure. Therefore, it is very unlikely that the effects of noise exposure on cortisol observed in the present study are mediated through sleep duration, psychiatric distress or annoyance. This could rather indicate that the smaller variation of cortisol levels over the day and the higher evening cortisol levels observed in this study are directly connected to aircraft noise exposure.

The 'airport' variable was not included in the final model in order to avoid over-adjustment. Indeed, when aircraft noise levels and the area of study were both introduced in the multivariate models, the results were not significant anymore. It is very likely due to over-adjustment, the effect of noise being distributed between both variables, and partly to a lack of statistical power. The results of the models including the study area instead of aircraft noise exposure lead to very similar conclusions than the ones including only aircraft noise exposure: the cortisol variation was lower in Paris where the aircraft noise levels were the highest than in Toulouse where they were at an intermediary level, than in Lyon where they were the lowest. But the 'airport' variable may also be associated with cortisol levels through other

characteristics than aircraft noise. Therefore, it is necessary to be careful with the association between cortisol secretion and aircraft noise exposure observed in the present study.

There was no association between aircraft noise exposure and cortisol variation or evening cortisol levels when exposure to road traffic noise was included in the models. However, estimation of exposure to road traffic noise based on noise maps was available only for 321 participants around Paris–Charles-de-Gaulle airport and was much less accurate compared to the one of aircraft noise exposure, thus reducing the statistical power to evidence any association between exposure to aircraft noise and cortisol if it was introduced in the models.

In this study, night-time and daytime exposure to aircraft noise at the place of residence were distinguished. Participants were more likely to be outside their home during the day than during the night, but no information was available about daytime aircraft noise exposure of the participants when outside their home, especially at their workplace. It was not possible to disentangle the effect on this variation of night-time exposure at home and daytime exposure at work. Misclassification of exposure might occur, but it is not likely that the exposure classification would depend on cortisol levels. Therefore, such non-differential misclassification would have induced an appreciable downward bias, if there is a true association between aircraft noise exposure and cortisol variation.

In this study, the modification of the cortisol circadian rhythm was significantly associated with day-evening-night and night-time exposures to aircraft noise. The decrease in cortisol variation was even higher with aircraft noise exposure during the night. Differences in the relationship between cortisol levels or variation and noise exposure regarding the use of different energy-based exposure indicators have never been studied in community noise research except in the HYENA study where a stronger association was found between morning cortisol levels and exposure to aircraft noise in the United Kingdom (Heathrow).[27]

Unlike airports in other countries with restrictions in night traffic, London's Heathrow is a major airport, with night flights, thus leading probably to higher night-time exposures to aircraft noise than in the other countries. Paris-Charles de Gaulle, Lyon Saint-Exupéry and Toulouse-Blagnac are three major French airports with night flights, even if there are some restrictions in night traffic: the noisiest aircrafts are not allowed to take off or to land between 22.00 and 6.00 in all the three airports. Despite the limitation of the number of flights between 0.00 and 5.00 in Paris-Charles de Gaulle, the number of night flights is much larger in this airport than in Lyon Saint-Exupéry and Toulouse-Blagnac, thus leading probably to higher night-time exposures to aircraft noise than in the other airports.

Two saliva samples during one sampling day were collected for each participant. These may be insufficient to reliably describe the circadian cortisol pattern.[41] However, because of the diurnal cortisol decrease, with an early morning maximum and a minimal level at night, it is reasonable to consider that a first sample after awakening and a second sample just before going to bed in the evening can give a reliable idea of cortisol diurnal variation. The distributions of cortisol levels were roughly right skewed even if it was less marked for morning saliva samples. The subjects were requested to collect the first sample immediately after awakening, but the samples might have been taken at different points during the first two hours after awakening. Moreover, the morning samples from all the participants spread between 5.00 am and 10.00 am, and the interval of normal values during this period of time is quite large compared with the one from evening samples.

It is also possible that diurnal cortisol variability may have affected baseline or reactive levels of cortisol. This issue was addressed by statistically controlling for the time of collection in the analyses when morning or evening cortisol levels were considered as the outcome variables or by dividing the cortisol variation by the number of hours between the two saliva

samples when cortisol variation per hour was considered as the outcome variable. As the time interval between the two measurements varied significantly between participants (from less than 6 hours to more than 14 hours), it was relevant to calculate the variation per hour in order to enable comparison between individuals. Perhaps, determining the same time sampling for all the participants would have been better, but it would have been more difficult to obtain a large compliance to the protocol.

Conclusions

The DEBATS study is the first to investigate the relationship between long-term aircraft noise exposure and saliva cortisol near French airports. The number of participants (n=954) included in this study was substantial compared to those included in other studies investigating this relation, and the participants followed sampling instructions very well. This study suggested a modification of the cortisol circadian rhythm in relation to aircraft noise exposure. This exposure was associated with a smaller variation of cortisol levels over the day, with unchanged morning cortisol levels, but higher cortisol levels in the evening. These findings provide an additional support for a psychological stress induced by aircraft noise, resulting in HPA dysregulation and a flattened cortisol rhythm, especially a lower ability to decrease cortisol levels at night in particular. Nevertheless, future studies should necessarily pay more attention to the interaction with other stressors.

Alteration in the cortisol rhythm has been associated with various negative outcomes like cardiovascular diseases. However, since the study of cortisol in large populations is a relatively new issue, epidemiological evidence contributing to explain the whole mechanism is still lacking.

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Competing interests

None.

Ethics approval

The present study was approved by two national authorities in France, the French Advisory Committee for Data Processing in Health Research and the French National Commission for Data Protection and the Liberties.

References

1 Marques AH, Silverman MN, Sternberg EM. Evaluation of stress systems by applying noninvasive methodologies: measurements of neuroimmune biomarkers in the sweat, heart rate variability and salivary cortisol. *Neuroimmunomodulation* 2010;17:205-8.

2 Horrocks PM, Jones AF, Ratcliffe WA et al. Patterns of ACTH and cortisol pulsatility over twenty-four hours in normal males and females. *Clinical Endocrinology* 1990;32:127-34.

3 Schmidt-Reinwald A, Pruessner JC, Hellhammer DH et al. The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences* 1999;64:1653-60.

4 Adam EK, Gunnar MR. Relationship functioning and home and work demands predict individual differences in diurnal cortisol patterns in women. *Psychoneuroendocrinology* 2001;26:189-208.

5 Hajat A, Diez-Roux A, Franklin TG et al. Socioeconomic and race/ethnic differences in daily salivary cortisol profiles: the multi-ethnic study of atherosclerosis. *Psychoneuroendocrinology* 2010;35:932-43.

6 Ranjit N, Young EA, Kaplan GA. Material hardship alters the diurnal rhythm of salivary cortisol. *International Journal of Epidemiology* 2005;34:1138-1143.

7 Desantis AS, Kuzawa CW, Adam EK. Developmental origins of flatter cortisol rhythms: socioeconomic status and adult cortisol activity. *American Journal of Human Biology* 2015;27:458-67.

8 Balbo M, Leproult R, Van Cauter E. Impact of sleep and its disturbances on hypothalamo-pituitary-adrenal axis activity. *International Journal of Endocrinology* 2010;2010:759234.

9 Wust S, Federenko I, Hellhammer DH, Kirschbaum C. Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology* 2000;25:707-20.

10 McEwen BS, Seeman T. Protective and damaging effects of mediators of stress - Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences* 1999;896:30-47.

11 Baum A, Grundberg G. *Measurement of stress hormones. In Measuring stress.* Eds Oxford University Press, New-York, 1995.

12 Evrard AS, Lefèvre M, Champelovier P, Lambert J, Laumon B. Does aircraft noise exposure increase the risk of hypertension in the population living near airports in France? . *Occupational and Environmental Medicine* 2017;74:123-129.

13 Jarup L, Babisch W, Houthuijs D et al. Hypertension and exposure to noise near airports: the HYENA study. *Environmental Health Perspectives* 2008;116:329-33.

14 Floud S, Vigna-Taglianti F, Hansell A et al. Medication use in relation to noise from aircraft and road traffic in six European countries: results of the HYENA study. *Occupational and Environmental Medicine* 2011;68:518-524.

15 Franssen EA, van Wiechen CM, Nagelkerke NJ, Lebret E. Aircraft noise around a large international airport and its impact on general health and medication use. *Occupational and Environmental Medicine* 2004;61:405-13.

16 Babisch W, van Kamp I. Exposure-response relationship of the association between aircraft noise and the risk of hypertension. *Noise & Health* 2009;11:161-8.

17 Eriksson C, Bluhm G, Hilding A, Ostenson CG, Pershagen G. Aircraft noise and incidence of hypertension - Gender specific effects. *Environmental Research* 2010;110:764-772.

18 Babisch W, Beule B, Schust M, Kersten N, Ising H. Traffic noise and risk of myocardial infarction. *Epidemiology* 2005;16:33-40.

19 Barregard L, Bonde E, Öhrström E. Risk of hypertension from exposure to road traffic noise in a population-based sample. *Occupational and Environmental Medicine* 2009;66:410-415.

20 Bluhm GL, Berglind N, Nordling E, Rosenlund M. Road traffic noise and hypertension. *Occupational and Environmental Medicine* 2007;64:122-126.

21 Hansell AL, Blangiardo M, Fortunato L et al. Aircraft noise and cardiovascular disease near Heathrow airport in London: small area study. *British Medical Journal* 2013;347:f5432.

22 Correia AW, Peters JL, Levy JI, Melly S, Dominici F. Residential exposure to aircraft noise and hospital admissions for cardiovascular diseases: multi-airport retrospective study. *British Medical Journal* 2013;347:f5561.

23 Evrard AS, Bouaoun L, Champelovier P, Lambert J, Laumon B. Does exposure to aircraft noise increase the mortality from cardiovascular disease of the population living in the vicinity of airports? Results of an ecological study in France. *Noise & Health* 2015;17:328-336.

24 Huss A, Spoerri A, Egger M, Röösli M. Aircraft Noise, Air Pollution, and Mortality From Myocardial Infarction. *Epidemiology* 2010;21:829-836.

25 Sorensen M, Hvidberg M, Andersen ZJ et al. Road traffic noise and stroke: a prospective cohort study. *European Heart Journal* 2011;32:737-744.

26 Babisch W. Stress Hormones in the Research on Cardiovascular Effects of Noise. *Noise & Health* 2003;5:1-11.

27 Selander J, Bluhm G, Theorell T et al. Saliva cortisol and exposure to aircraft noise in six European countries. *Environmental Health Perspectives* 2009;117:1713-7.

28 Kirschbaum C, Hellhammer DH. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. *Psychoneuroendocrinology* 1994;19:313-33.

29 Stokholm ZA, Hansen AM, Grynderup MB et al. Recent and long-term occupational noise exposure and salivary cortisol level. *Psychoneuroendocrinology* 2014;39:21-32.

30 Basner M, Müller U, Griefahn B. Practical guidance for risk assessment of traffic noise effects on sleep. *Applied Acoustics* 2010;71:518-522.

31 Wilhelm I, Born J, Kudielka BM, Schlotz W, Wust S. Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology* 2007;32:358-66.

32 Abercrombie HC, Giese-Davis J, Sephton S, Epel ES, Turner-Cobb JM, Spiegel D. Flattened cortisol rhythms in metastatic breast cancer patients. *Psychoneuroendocrinology* 2004;29:1082-92.

33 Cohen S, Schwartz JE, Epel E, Kirschbaum C, Sidney S, Seeman T. Socioeconomic status, race, and diurnal cortisol decline in the Coronary Artery Risk Development in Young Adults (CARDIA) Study. *Psychosomatic Medicine* 2006;68:41-50.

34 Rich EL, Romero LM. Exposure to chronic stress downregulates corticosterone responses to acute stressors. *American Journal of Physiology-Regulatory Integrative and Comparative Physiology* 2005;288:R1628-R1636.

35 Dmitrieva NO, Almeida DM, Dmitrieva J, Loken E, Pieper CF. A day-centered approach to modeling cortisol: Diurnal cortisol profiles and their associations among U.S. adults. *Psychoneuroendocrinology* 2013;38:2354-2365.

36 Wood SN. *Generalized Additive Models: An Introduction with R*. London: Chapman and Hall/CRC Press, 2006.

37 Zimmer C, Basler HD, Vedder H, Lautenbacher S. Sex differences in cortisol response to noxious stress. *Clinical Journal of Pain* 2003;19:233-239.

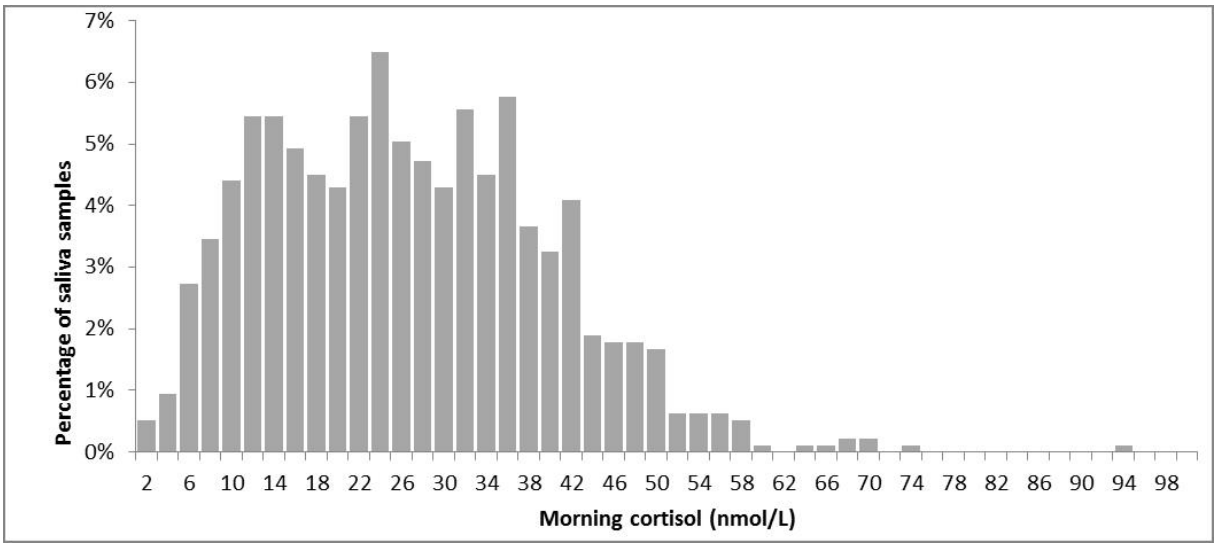
38 Paris JJ, Franco C, Sodano R et al. Sex differences in salivary cortisol in response to acute stressors among healthy participants, in recreational or pathological gamblers, and in those with posttraumatic stress disorder. *Hormones and Behavior* 2010;57:35-45.

39 Kirschbaum C, Wust S, Hellhammer D. Consistent sex differences in cortisol responses to psychological stress. *Psychosomatic Medicine* 1992;54:648-57.

40 Kudielka BM, Hellhammer DH, Wust S. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 2009;34:2-18.

41 Bigert C, Bluhm G, Theorell T. Saliva cortisol - a new approach in noise research to study stress effects. *International Journal of Hygiene and Environmental Health* 2005;208:227-230.

(A)



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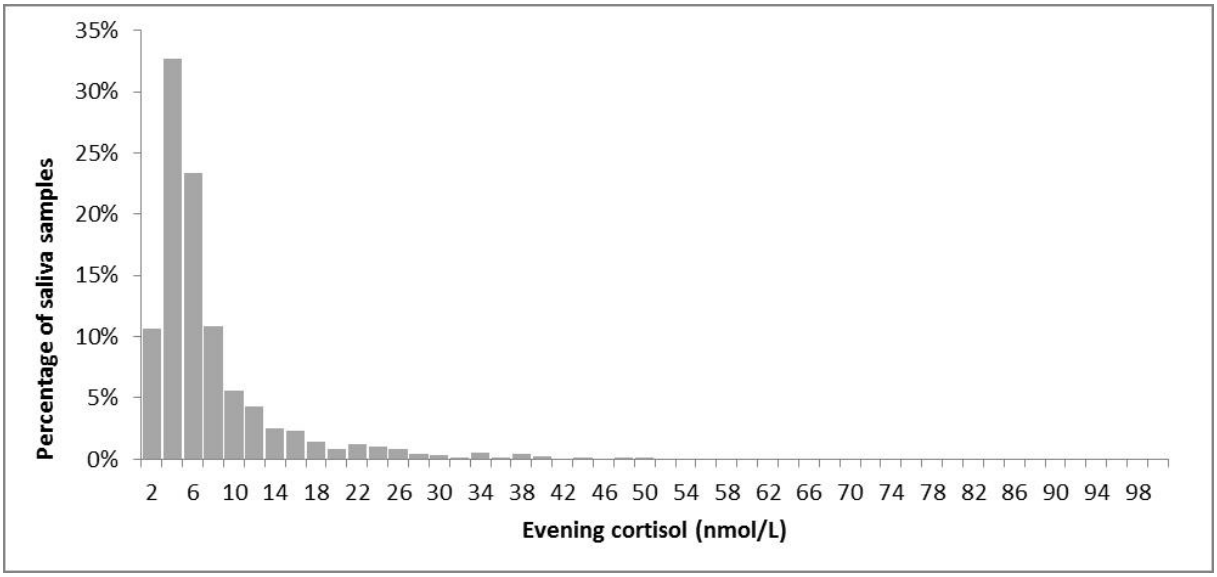

Figure 1. (A) Distribution of cortisol for morning saliva samples from the 954 participants. (B) Distribution of cortisol for evening saliva samples from the 954 participants.

Table 1: Number of participants providing saliva samples in the DEBATS study and included in the cortisol analyses, by airport and noise exposure at residence

	Aircraft noise exposure [L_{den} (dB(A))]				No. of
Airport	< 50	50 - 54	55 - 59	≥ 60	subjects
Paris-Charles de Gaulle	80	76	144	157	457
Toulouse-Blagnac	85	78	74	80	317
Lyon Saint-Exupéry	89	85	5	1	180
Total	254	239	223	238	954

Table 2: Average sampling time and cortisol levels across the four aircraft noise levels (954 subjects)

	Aircraft noise exposure [L_{den} (dB(A))]				
	< 50	50 - 54	55 - 59	≥ 60	Total
Average sampling time (standard deviation)					
At awakening	07.28 (01.02)	07.32 (1.09)	07.28 (1.03)	07.24 (1.07)	07.28 (1.05)
Before going to bed	22.46 (5.52)	22.47 (4.55)	22.45 (5.16)	22.45 ((.44)	22.46 (5.28)
Average cortisol level in saliva in nmol/L (standard deviation)					
Variation per hour	2.3 (1.5)	2.4 (1.5)	2.2 (1.6)	2.0 (1.6)	2.2 (1.5)
At awakening	25.4 (12.7)	26.2 (12.4)	26.2 (13.6)	24.6 (14.2)	25.6 (13.2)
Before going to bed	5.9 (5.4)	6.5 (7.6)	6.7 (6.5)	7.2 (6.4)	6.6 (6.5)

Table 3: Linear regression coefficients after exponentiation for the relation between cortisol variation per hour and the major a priori confounders

Variable	exp(β)[a] (95% CI)	exp(β)[a] (95% CI)
Sampling day		
Weekend	1.00	1.00
Weekday	**1.47 (1.19-1.81)**	**1.44 (1.17-1.78)**
Age	1.00 (1.00-1.00)	1.00 (1.00-1.01)
Gender		
Female	1.00	1.00
Male	1.06 (0.91-1.23)	1.05 (0.91-1.22)
BMI	0.99 (0.98-1.01)	0.99 (0.98-1.01)
Physical Activity		
No	1.00	1.00
Yes	**1.32 (1.13-1.53)**	**1.30 (1.12-1.51)**
Household monthly income		
< 2,300 euros (2,600 US$)	**0.79 (0.66-0.95)**	**0.82 (0.68-0.99)**
2,300 - 4,000 euros (2,600 – 4,500 US$)	0.92 (0.77-1.10)	0.93 (0.78-1.12)
≥ 4,000 euros (4,500 US$)	1.00	1.00
Alcohol consumption		
No	1.00	1.00
Light	1.09 (0.92-1.29)	1.08 (0.91-1.28)
Moderate	1.18 (0.94-1.48)	1.18 (0.94-1.48)
Heavy	1.27 (0.88-1.85)	1.25 (0.87-1.82)

Variable	exp(β)[a] (95% CI)	exp(β)[a] (95% CI)
Smoking habits		
Non smoker	1.00	1.00
Ex smoker	1.10 (0.92-1.30)	1.11 (0.94-1.32)
Occassional smoker	0.96 (0.48-1.91)	0.98 (0.49-1.96)
Daily smoker	0.96 (0.79-1.16)	0.97 (0.80-1.18)
Psychiatric disorder		
No disorder	-	1.00
Moderate disorder	-	1.04 (0.88-1.23)
Severe disorder	-	0.79 (0.54-1.15)
Sleep duration		
≤ 5h	-	0.63 (0.39-1.01)
6h	-	1.02 (0.80-1.30)
7h	-	1.00
8h	-	1.04 (0.87-1.24)
≥ 9h	-	**0.81 (0.66-0.99)**
Annoyance		
Not annoyed	-	1.00
Annoyed	-	0.94 (0.81-1.08)

All possible confounding factors were included simultaneously in the model.

Bold values are statistically significant $p \leq 0.05$.

[a] An exponentiation was applied on the log-transformed estimates. The results can be directly interpreted as relative risks.

Table 4: Linear regression coefficients after exponentiation for the relation between cortisol variation per hour, morning or evening saliva cortisol levels and aircraft noise exposure.

	Cortisol variation per hour			**Morning levels**			**Evening levels**		
	M0	M1	M2	M0	M1	M2	M0	M1	M2
Noise indicator (dBA)[b]	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)	exp(β)[a] (95%CI)
L_{den}	**0.81 (0.72-0.91)**	**0.84 (0.74-0.95)**	**0.85 (0.75-0.96)**	0.98 (0.92-1.05)	0.99 (0.93-1.07)	1.00 (0.92-1.08)	**1.18 (1.09-1.29)**	**1.15 (1.05-1.25)**	**1.17 (1.07-1.28)**
$L_{Aeq,24h}$	**0.82 (0.72-0.93)**	**0.84 (0.74-0.97)**	**0.86 (0.74-0.99)**	1.00 (0.92-1.08)	1.01 (0.93-1.09)	1.01 (0.93-1.10)	**1.18 (1.08-1.30)**	**1.15 (1.04-1.26)**	**1.17 (1.06-1.29)**
$L_{Aeq,16h}$	**0.84 (0.75-0.94)**	**0.86 (0.77-0.97)**	**0.87 (0.77-0.99)**	1.00 (0.93-1.07)	1.00 (0.94-1.08)	1.01 (0.94-1.08)	**1.16 (1.07-1.26)**	**1.13 (1.04-1.22)**	**1.15 (1.05-1.25)**
L_{night}	**0.79 (0.71-0.88)**	**0.82 (0.74-0.92)**	**0.83 (0.74-0.93)**	0.96 (0.90-1.03)	0.98 (0.92-1.05)	0.98 (0.92-1.05)	**1.18 (1.10-1.28)**	**1.15 (1.07-1.25)**	**1.17 (1.08-1.27)**

Bold values are statistically significant $p \leq 0.05$.

M0: model including only aircraft noise exposure.

M1: model including aircraft noise exposure, the day of the week of the cortisol sampling, gender, age, BMI, physical activity, household monthly income, alcohol consumption, and smoking habits. Analyses on morning and evening cortisol levels included also the sampling time.

M2: model including aircraft noise exposure, the day of the week of the cortisol sampling, gender, age, BMI, physical activity, household monthly income, alcohol consumption, smoking habits, psychiatric disorders, sleep duration and aircraft noise annoyance. Analyses on morning and evening levels included also the sampling time