

The relationship between smoking status and cortisol secretion.

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Key terms – smoking, salivary cortisol, epidemiology, Whitehall II, HPA axis,

## **Abstract**

Context: Evidence for an association of smoking status with cortisol secretion is mixed.

Objective: To assess the relationship between smoking status and salivary cortisol.

Design: Cross sectional study of smoking status and cortisol secretion from phase 7 (2002-2004) of the Whitehall II study.

Setting: Occupational cohort originally recruited in 1985-1987.

Participants: 3103 men (1514 never smokers, 1278 ex-smokers, and 311 smokers) and 1128 women (674 never smokers, 347 ex-smokers and 107 smokers). Information was collected on smoking status, average number of cigarettes smoked, and additional covariates.

Outcome measures: Saliva samples were taken on waking, waking + 0.5, 2.5, 8, 12 hours and bedtime for the assessment of cortisol.

Results: Smoking status was significantly associated with increased salivary cortisol release throughout the day ( $p < 0.001$ ) adjusted for covariates, this was apparent for the cortisol awakening response (CAR) ( $p < 0.001$ ) when examined separately. Compared to never smokers, smokers had higher release of total cortisol,  $p = 0.002$ , while no difference was observed between never smokers and ex-smokers ( $p = 0.594$ ), [mean release per hour nmol/l: never smokers 4.13 (CI: 4.02-4.24), ex-smokers 4.21 (CI: 4.08-4.35), smokers 4.63 (CI: 4.35-4.93)]. There was no significant relationship between number of cigarettes smoked and total cortisol release. However, a difference was observed for the CAR: mean release by tertiles of cigarettes smoked (nmol/l): high 13.49 (CI: 10.74-16.23), medium 9.58 (CI: 7.40-11.76), low 8.49 (CI: 5.99-10.99)  $p = 0.029$ .

Conclusion: Salivary cortisol is increased in current smokers compared with non-smokers, no differences were observed between ex-smokers and never smokers suggesting that smoking has a short-term affect on the neuroendocrine system.

## **Introduction**

The detrimental effect of smoking on health is well documented with studies predominantly focusing on coronary heart disease and cancer (1). Recent reviews of the effects of smoking on endocrine function document that smoking has multiple effects on hormone secretion including effects on the hypothalamic pituitary adrenal axis (HPA axis) (2;3). The HPA axis is primarily used in the body's response to physical and mental stress (4). A reliable biological marker for this response is increased peripheral cortisol concentration. It is hypothesized that prolonged activation of this axis can be detrimental to health, and may provide a link between mental stress and physical illness (5). Activation of the HPA axis is implicated in the aetiology of a number of diseases including osteoporosis and heart disease (6).

Studies have also shown a direct acute affect of smoking on cortisol levels (7), effects which have been attributed to activation of central nicotinic receptors. Activation of the HPA axis is reported to require acute nicotine exposure (consumption of two high strength cigarettes in quick succession), and some studies have shown a dose response action (8-10). It is believed the HPA axis is altered in smokers (11); however, the reported effects of habitual smoking on cortisol levels in everyday life are mixed with some studies indicating no relationship (12-14) while others report that smoking is associated with increased cortisol secretion (15-19). The differences in the findings of these studies may be attributed to small study sample (19), differences in sample design (measurement of cortisol in urine or plasma), and differences in timing of the sample collection (15)

Cortisol has a diurnal profile which is characterised by a substantial increase in cortisol secretion following awakening, peaking at about 30 minutes, and a subsequent decline over the remainder of the day. It is hypothesized that the CAR and release across the day are under different control mechanisms. There is evidence that the cortisol awakening response (CAR) is greater in smokers compared to non-smokers (19) and smokers have greater cortisol production in assessment of samples over the rest of the day. (15).

Cortisol can be measured in saliva, urine or plasma. The use of saliva sampling allows reliable unobtrusive measurement in a naturalistic setting, and can be designed to capture the diurnal profile of cortisol (20;21). The collection of salivary cortisol is not awkward for participants or subject to white coat syndrome and a person can participate in their 'normal' daily activities.

We wish to examine the association between smoking status and salivary free cortisol secretion in a large cohort of middle-aged men and women. We hypothesize that cortisol released throughout the day will be raised in smokers compared never smokers and ex-smokers. We will investigate a possible dose response action by assessing cortisol release of smokers by number of cigarettes smoked. We will examine differences in the cortisol awakening response and cortisol release throughout the day. Our study has the advantage of being large enough to take into account correlates of cortisol secretion in the analysis, and allow us to assess differences between never, ex and current smokers.

## **Subjects and Methods**

**Participants:** Data reported here are from phase 7 (2002-2004) of the Whitehall II study. The Whitehall II cohort was initially recruited between 1985 to 1988 (phase 1) from 20 London based civil service departments, 10308 people participated, details of the clinical assessment and cohort profile have been reported elsewhere (22). The number participating at phase 7 was 6941, of these 93.4% had a clinical assessment, of those eligible for cortisol assessment 90.1% (n=4609) returned samples. In analyses reported here fewer participants were in the lowest grades compared to phase 1 of the study, however this difference was small. Ethical approval for the Whitehall II study was obtained from the University College London Medical School committee on the ethics of human research. Informed consent for involvement in the study was gained from every participant.

**Cortisol collection and analysis:** To collect a saliva sample participants used a device called a salivette (Sarstedt, Leicester, UK). Participants were instructed to provide six samples over the course of a normal weekday at waking, waking +30mins, waking +2.5hours, waking +8hours, waking +12hours and bedtime. Participants were instructed to record the time of sample collection, take samples as soon as they woke, to avoid caffeine and acidic drinks in the first 30 minutes. Not to brush teeth, eat or drink anything for 15 minutes prior to a sample collection. An instruction booklet was given that acted as a logbook to record information on wake time, mood at time of sampling, smoking, alcohol consumption, exercise and stressful events on the day of sampling. The salivettes and logbook were returned via post. Salivette devices were centrifuged at 3,000 rpm for 5 minutes resulting in a clear supernatant of low viscosity. Salivary cortisol levels were

measured using a commercial immunoassay with chemiluminescence detection (CLIA, IBL-Hamburg, Hamburg, Germany). The lower concentration limit of this assay is 0.44 nmol/l; intra and interassay coefficients of variance were below 8%. Any sample over 50µg was repeated.

**Smoking** status was assessed by the following questions 1) ‘Do you smoke cigarettes now (that is not cigars/pipe)?’ with responses ‘yes’, ‘no’ or ‘social/occasional smoker’, 2) ‘If not a current cigarette smoker, did you smoke in the past?’ and 3) ‘Do you currently smoke cigars or a pipe?’ with ‘yes’ or ‘no’ response options. We used the logbook question ‘Did you use any tobacco today? (e.g., cigarettes, cigars, pipes, chewing tobacco)’ yes or no. From these questions a person was assigned to be a current, ex or never smoker, if any nicotine replacement therapy was used they were assigned a smoker (n=4). For ex-smokers the response to ‘How old were you when you stopped smoking?’ with their age at the screening was used to calculate the length of time since quitting. Participants were asked to record how many cigarettes they smoked on the day of sampling, this was categorized into tertiles of low, medium and high numbers of cigarettes smoked. This was calculated separately for men and women resulting in cut points of 6 and 15 cigarettes for men, and 8 and 15 cigarettes for women.

**Covariates:**

Social position was determined by job grade if still in the civil service, or based on last grade if they had left the service. For our analysis civil service grades are collapsed into 3 categories. Low income was defined as earning less than £15,000 a year. Wealth was assessed by the question ‘If you sold all your assets your household owns for example [*list of assets*] and cashed in savings and investments and paid off all your debts

(including your mortgage) how much money do you think you would have?' low wealth defined as between £0-£99,000. More details on the definitions of income and wealth can be found in Martikainen et al 2003 (23).

Financial insecurity was assessed using the question 'Thinking of the next ten years how financially secure do you feel?' with secure, fairly secure, fairly insecure and insecure the possible answers. Participants responding feeling insecure or fairly insecure were defined as feeling financial insecure, as in Ferrie et al (2003) (24).

Depression was measured using the General Health Questionnaire (GHQ) depression subscale using four items, respondents scoring 0–2 were considered "non-cases" and those scoring 3 or more, "GHQ depression cases". This is not a clinical definition but one validated to be used for the 30-item GHQ scale (25).

Diabetes was assessed if participants stated they were diabetic when asked, or a post load glucose  $\geq 11.1$ mmol/l ( if missing a fasting glucose  $\geq 7.0$  mmol/l)

Poor Health was assessed by the question 'In general would you say your health is -' five response items Excellent, Very good, Good, Fair or Poor. 'Fair' or 'poor' were categorized as having poor health.

Alcohol consumption was calculated using the units consumed in a week, high alcohol intake was assigned if greater than 21 units for men and 14 for women. The logbook provided alcohol consumed on the day of sample collection 'did you drink alcoholic

beverages today?’ Number of alcoholic drinks was recorded, high daily alcohol consumption was defined as > 4 drinks a day for men and >3 drinks a day for women.

Body mass index (BMI) was assessed by measurement of height and weight in the standard way at the clinical assessment. BMI was calculated as weight/height squared.

Stress on the day of sample collection was assessed with the question ‘We’d like to know if this was a typical day for you, compared to your usual workdays (or weekends), in terms of how busy, pressured, or stressed you felt.’ response options were ‘Today was typical/greater/lower in terms of my workload or stress level’. Participants were asked about the most stressful event and categories were ‘not at all stressed’, ‘somewhat stressed’, ‘moderately stressed’, ‘very stressed’ or ‘the most stressed I have ever felt’. Participants classified as having a stressful experience if they responded that they were ‘very stressed’ or ‘the most stressed I have ever felt’.

### **Statistical techniques:**

Gender has been shown to have an effect on smoking status (26) and cortisol levels. However, there was no interaction between smoking status and gender, following adjustment for age and last known employment grade, analyses were therefore combined. Participant characteristics by smoking status were examined by chi squared analysis for categorical variables and linear regression for continuous variables. Due to non-normal distribution cortisol values were log transformed for analysis, normal data are presented in figures. For each of the cortisol samples outliers were removed (sample 1 n=4, sample 2 n=5, sample 3 n=5, sample 4 n=4, sample 5 n=4, sample 6 n=3). For missed samples imputations were performed for samples taken at time 3 (n=16), 4 (n=37), and 5 (n=36).

No statistical differences were observed therefore imputed results are presented. The cortisol release over the course of the day was analyzed using a general linear model for repeated measures. The CAR is calculated as the ratio of cortisol in sample 1 to cortisol in sample 2.

Analyses examining the association between smoking status and cortisol secretion were first run with adjustment for age, gender and employment grade as a measure of social position only. Effect sizes were calculated to determine the size of the association. Models were then run with additional adjustments for other potential confounding or mediating factors. These analyses included participants with complete data on all variables included in the models. The association with number of cigarettes smoked and cortisol secretion was also assessed by regression. All data were analysed using SPSS version 13.0, Bonferroni correction was used for multivariate analysis

## Results

From the samples returned 168 individual samples were not taken by participants which equates to 0.55% of the total number of samples expected. For technical reasons, 1002 samples were not assayed. Participants taking medications affecting cortisol levels were removed from the analysis (n=236). The final number of participants with reliable cortisol measurement is 3103 men (1514 never smokers, 1278 ex-smokers, and 311 smokers) and 1128 women (674 never smoked, 347 ex-smokers and 107 smokers). Studies have shown that a delay in taking sample 1 results in a reduced CAR, as the morning cortisol peak is already substantially underway (27). Analysis was conducted on samples that were taken within 10 minutes of waking (sample 1 taken >10 minutes n= 634). Participants were excluded if they recorded that they ate, drank, exercised or brushed their teeth before the first sample (n=41). For the total population that participated at phase 7 (n=6940) the distribution of smoking status is never smokers 52.7%, ex-smokers 38.1% and smokers 9.2% the distribution is not significantly different from the participants with reliable cortisol measures. Men smoked on average 11.7 cigarettes and women 12.7 cigarettes on the day of saliva collection. For men the ex-smokers had been abstinent for an average of 26.3 years and women for 24.5 years.

The participant characteristics by smoking status are shown in table 1. Smokers were younger (ANOVA  $p<0.001$ ) more likely to be in the lower employment grades ( $p<0.001$ ), lowest income ( $p=0.008$ ), and lowest wealth ( $p=<0.001$ ) groups. Smokers had a higher weekly alcohol consumption ( $p<0.001$ ) and higher consumption of alcohol on the day of sampling ( $p<0.001$ ), and more likely to have a BMI > 30 ( $p=0.02$ ). Smokers reported

poorer self-rated health ( $p < 0.001$ ), more depressive symptoms ( $p = 0.02$ ), and felt more financially insecure ( $p < 0.001$ ).

The profiles for cortisol release over the course of the day are illustrated in Figure 1; smokers had a larger production of cortisol over the course of the day in repeated measures analysis  $p < 0.001$ . This is confirmed when total cortisol production over the day is examined (overall ANOVA  $p = 0.008$ ) see Figure 2. Cortisol secretion was higher in smokers compared to never smokers ( $p = 0.002$ ) and no significant difference observed between never and ex-smokers ( $p = 0.594$ ).

Table 2 illustrates that the increased release of cortisol in smokers compared to never smokers which is robust to adjustment, thus cortisol levels were raised in smokers [mean release per hour in nmol/l/h: 4.64 (CI: 4.36-4.95)]  $p = 0.005$  for between subjects effects in a repeated measures analysis of variance. No difference was observed between never smokers [4.14 (CI: 4.03-4.26)] and ex-smokers [4.20 (CI: 4.07-4.34),  $p = 0.594$ ] following adjustment for age, gender, employment grade, BMI, high alcohol consumption, poor health, depression and financial insecurity. The effect size between smokers and never smokers was 0.28. The CAR was larger in smokers  $p < 0.001$  (adjusted for age, gender, grade and time of waking), smokers CAR was 9.73 (CI: 8.42-11.04) nmol/l compared to never smokers 7.52 (CI: 6.96-8.09) nmol/l. This effect size was 0.15. In assessment of the CAR, time of waking  $p < 0.001$ , and gender  $p = 0.012$  had independent relationships with the size of CAR.

The dose response relationship for numbers of cigarettes smoked and cortisol release is illustrated in Figure 3. There is no relationship between tertiles of number of cigarettes

smoked and release of cortisol in the course of the day [repeated measures assessment of cortisol ( $p=0.646$ ) or mean cortisol produced over the day ( $p=0.095$ )]. The CAR was assessed by increasing tertile of cigarettes smoked [low 8.49 (CI: 5.99-10.99) medium 9.58(CI: 7.40-11.76) and high 13.49(CI: 10.74-16.23)] and shows a positive trend ( $p=0.029$ ). Linear regression was performed for number of cigarettes smoked on the day including age, gender and last known grade as covariates, with mean cortisol per hour of the day as the dependant variable. There was no significant relationship with number of cigarettes smoked ( $p=0.231$ ,  $b=1.078$ ,  $n=270$ ) and mean cortisol.

Inclusion of never smokers as the comparison group in repeat measures analysis comparison to each tertile of cigarettes smoked: low  $p=0.117$ , medium  $p=0.028$  and high  $p=0.006$ . In ANOVA of mean cortisol produced over the day using never smokers as the comparison group, Figure 4, difference is only significant for high tertile consumption group (low  $p=0.416$ , medium  $p=0.417$ , high  $p=0.001$ ). Linear regression analysis suggested that there was a linear association between tertiles of cigarettes smoked and total cortisol secretion ( $\beta= 1.07$ ,  $p=0.003$ ) following adjustment for age, gender and last known grade.

Examination of time since quitting in ex-smoking group showed no differences for length of time abstinent, for any of the cortisol measures (data not shown).

## **Discussion**

We have shown that in a large population study of middle-aged men and women, smoking is associated with raised cortisol secretion throughout the day. This effect is observed for assessment of total cortisol production over the day and when the cortisol awakening response and release throughout the rest of the day are examined separately. These effects are independent of gender, social status, health behaviors and stress reporting. There is an association between number of cigarettes smoked and the CAR but not other measures of cortisol production.

These results support some (15;27) but not all (13, 28) previous reports of an association between cigarette smoking and increased cortisol secretion. These findings may reflect methodological differences in study design or differences in sample size. The size of the study allowed comparisons to be made with ex-smokers in relation to never and current smokers. Two lines of evidence suggest that the effect of smoking on the HPA axis is not due to the chronic effect of smoking on health, firstly once smoking has ceased the diurnal pattern of cortisol release returns to that of a never smoker and secondly adjustment for health measures failed to alter our results. Investigation of the dose response association with numbers of cigarettes indicates that the differences are driven largely by the heavy smoking group, supporting evidence that smoking has a short-term effect on the HPA axis.

The effect size of smoking on cortisol secretion is small to moderate (0.28) but this consistent effect every day over many years may potentially have large consequences on downstream endocrine function. For example, evidence suggests that the moderately

raised cortisol levels observed in smokers would potentially have large impacts on glucose and insulin metabolism(28;29), and small increases in cortisol are associated with reduced bone mineral density (30), this is a potential link between smoking and development of osteoporosis.

The potential pathways by which smoking affects the HPA axis are numerous and the literature is unclear about the mechanisms that could be mediating the effect. Activation of central nicotinic receptors to raise systemic cortisol levels has been posited, however this effect is not observed in users of nicotine replacement, cortisol is usually decreased in people attempting to quit (31). There is evidence of desensitization to the effects of nicotine in animal models (32); however it is unclear whether this applies for humans especially if they have been smoking for over 40 years. The mode of ingestion of nicotine could be relevant in activation of receptors or the influence of other chemicals and compounds in cigarette smoke that activate the HPA axis. If this were the case it is unclear why we observed differences in the CAR rather than release throughout the day. Evidence indicates that nicotine diminishes in the body within a few hours but other metabolites of smoking are present for longer periods (days) (33) and these may mediate the changes in the CAR. Alternately, an immune action induced by inflammation of the lung tissue due to smoke inhalation, or more generalized effect mediated by low systemic inflammation could explain the increase cortisol for smokers observed for the CAR and measures over the rest of the day. The final possibility is there is no direct affect of smoking on the HPA axis it is confounding by other social factors or underlying illness not accounted for in these analyses.

The positive and negative aspects of our study need to be considered. The percentage of smokers in this study is smaller than that of other studies (15), however the overall size of the study is greater, it had an excellent response rate and has allowed assessment of the HPA axis in ex-smokers. Indicators suggest that the participants understood the instructions, and took samples in the correct manner confirming the reliability of the dataset. A large number of possible confounders are measured and adjusted for, the results are still significant. However, these are cross-sectional analyses that make it difficult to assess the direction of causation. Thus it is possible that a stressful environment raises cortisol levels and encourages people to smoke or continue to smoke. This latter theory is not well supported by the literature. The Whitehall II study is an occupational cohort of UK civil servants and therefore may not be representative of the population, which may reduce the generalizability of the results. The rate of smoking in this cohort is 9.2%, in the UK population it is 25% in this age group (34). The effect smoking has on cortisol levels means the smaller number of smokers in this population does not affect the ability to detect HPA axis changes in response to smoking. We are unable to determine the length of time between consumption of a cigarette and when a saliva sample was taken. We believe that knowledge of this information would not change our overall conclusion that there are no long term effects of smoking on cortisol secretion. Smokers are known to have poorer oral health (35), and leakage of plasma cortisol into the saliva could artificially raise salivary cortisol levels in smokers. Evidence suggests blood leakage into the saliva can have a slight effect on cortisol levels (D. Granger personal communication), however oral micro injury has been shown not to significantly alter salivary cortisol levels (36), and studies showing increased plasma cortisol in smokers would dispute this theory (15). There were too few participants taking

nicotine replacements to assess the role of nicotinic activation compared to other chemicals in cigarette smoke and inflammatory activation due to smoke inhalation.

In conclusion, these results indicate that activation of the HPA axis occurs in smokers and affects diurnal rhythm of cortisol as illustrated by differences observed for the CAR and whole day assessment. The mechanisms by which these associations occur remain to be determined but appear not to involve confounding by social position or mediated by psychosocial stress pathways. The large group of ex-smokers available enabled comparison of this group and no differences were observed with never smokers indicating no long-term effect of smoking on the HPA axis.

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## Reference List

1. **Doll R, Peto R, Boreham J, Sutherland I** 2005 Mortality from cancer in relation to smoking: 50 years observations on British doctors. *Br J Cancer* 92:426-429
2. **Kapoor D, Jones TH** 2005 Smoking and hormones in health and endocrine disorders. *Eur J Endocrinol* 152:491-499
3. **Tziomalos K, Charsoulis F** 2004 Endocrine effects of tobacco smoking. *Clin Endocrinol (Oxf)* 61:664-674
4. **Miller DB, O'Callaghan JP** 2002 Neuroendocrine aspects of the response to stress. *Metabolism* 51:5-10
5. **Kristenson M, Eriksen HR, Sluiter JK, Starke D, Ursin H** 2004 Psychobiological mechanisms of socioeconomic differences in health. *Soc Sci Med* 58:1511-1522
6. **Manelli F, Giustina A** 2000 Glucocorticoid-induced osteoporosis. *Trends Endocrinol Metab* 11:79-85
7. **Chiodera P, Volpi R, Capretti L, Speroni G, Necchi-Ghiri S, Caffarri G, Colla R, Coiro V** 1997 Abnormal effect of cigarette smoking on pituitary hormone secretions in insulin-dependent diabetes mellitus. *Clin Endocrinol (Oxf)* 46:351-357
8. **Pomerleau OF** 1992 Nicotine and the central nervous system: biobehavioral effects of cigarette smoking. *Am J Med* 93:2S-7S
9. **del Arbol JL, Munoz JR, Ojeda L, Cascales AL, Irlles JR, Miranda MT, Ruiz Requena ME, Aguirre JC** 2000 Plasma concentrations of beta-endorphin in smokers who consume different numbers of cigarettes per day. *Pharmacol Biochem Behav* 67:25-28
10. **Mendelson JH, Sholar MB, Goletiani N, Siegel AJ, Mello NK** 2005 Effects of low- and high-nicotine cigarette smoking on mood states and the HPA axis in men. *Neuropsychopharmacology* 30:1751-1763
11. **Rohleder N, Kirschbaum C** 2006 The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. *Int J Psychophysiol* 59:236-243
12. **Yeh J, Barbieri RL** 1989 Twenty-four-hour urinary-free cortisol in premenopausal cigarette smokers and nonsmokers. *Fertil Steril* 52:1067-1069
13. **Cherek DR, Smith JE, Lane JD, Brauchi JT** 1982 Effects of cigarettes on saliva cortisol levels. *Clin Pharmacol Ther* 32:765-768
14. **Anthenelli RM, Maxwell RA** 2002 Independent alcohol and tobacco effects on stress axis function. *Alcohol Clin Exp Res* 26:1932-1933

15. **Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB** 1994 The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. *J Clin Endocrinol Metab* 79:1310-1316
16. **Baron JA, Comi RJ, Cryns V, Brinck-Johnsen T, Mercer NG** 1995 The effect of cigarette smoking on adrenal cortical hormones. *J Pharmacol Exp Ther* 272:151-155
17. **al'Absi M, Wittmers LE, Erickson J, Hatsukami D, Crouse B** 2003 Attenuated adrenocortical and blood pressure responses to psychological stress in ad libitum and abstinent smokers. *Pharmacol Biochem Behav* 74:401-410
18. **Kirschbaum C, Wust S, Strasburger CJ** 1992 'Normal' cigarette smoking increases free cortisol in habitual smokers. *Life Sci* 50:435-442
19. **Steptoe A, Ussher M** 2006 Smoking, cortisol and nicotine. *Int J Psychophysiol* 59:228-235
20. **Smyth JM, Ockenfels MC, Gorin AA, Catley D, Porter LS, Kirschbaum C, Hellhammer DH, Stone AA** 1997 Individual differences in the diurnal cycle of cortisol. *Psychoneuroendocrinology* 22:89-105
21. **Kirschbaum C, Hellhammer DH** 1989 Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 22:150-169
22. **Marmot M, Brunner E** 2005 Cohort Profile: the Whitehall II study. *Int J Epidemiol* 34:251-256
23. **Martikainen P, Adda J, Ferrie JE, Davey SG, Marmot M** 2003 Effects of income and wealth on GHQ depression and poor self rated health in white collar women and men in the Whitehall II study. *J Epidemiol Community Health* 57:718-723
24. **Ferrie JE, Shipley MJ, Stansfeld SA, Smith GD, Marmot M** 2003 Future uncertainty and socioeconomic inequalities in health: the Whitehall II study. *Soc Sci Med* 57:637-646
25. **Stansfeld SA, Head J, Marmot MG** 1998 Explaining social class differences in depression and well-being. *Soc Psychiatry Psychiatr Epidemiol* 33:1-9
26. **Bolego C, Poli A, Paoletti R** 2002 Smoking and gender. *Cardiovasc Res* 53:568-576
27. **Kudielka BM, Broderick JE, Kirschbaum C** 2003 Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosom Med* 65:313-319
28. **Spiegel K, Leproult R, Van Cauter E** 1999 Impact of sleep debt on metabolic and endocrine function. *Lancet* 354:1435-1439
29. **Plat L, Leproult R, L'Hermite-Baleriaux M, Fery F, Mockel J, Polonsky KS, Van Cauter E** 1999 Metabolic effects of short-term elevations of plasma cortisol

are more pronounced in the evening than in the morning. *J Clin Endocrinol Metab* 84:3082-3092

30. **Raff H, Raff JL, Duthie EH, Wilson CR, Sasse EA, Rudman I, Mattson D** 1999 Elevated salivary cortisol in the evening in healthy elderly men and women: correlation with bone mineral density. *J Gerontol A Biol Sci Med Sci* 54:M479-M483
31. **Ussher M, West R, Evans P, Steptoe A, McEwen A, Clow A, Hucklebridge F** 2006 Reduction in cortisol after smoking cessation among users of nicotine patches. *Psychosom Med* 68:299-306
32. **Wang H, Sun X** 2005 Desensitized nicotinic receptors in brain. *Brain Res Brain Res Rev* 48:420-437
33. **Hecht SS, Carmella SG, Chen M, Dor Koch JF, Miller AT, Murphy SE, Jensen JA, Zimmerman CL, Hatsukami DK** 1999 Quantitation of urinary metabolites of a tobacco-specific lung carcinogen after smoking cessation. *Cancer Res* 59:590-596
34. **McMunn A, Hyde M, Janevic M, Kumari M** 2002 Health, wealth and lifestyles of the older population in England: the 2002 English Longitudinal Study of Ageing. Institute of Fiscal Studies, London
35. **Taybos G** 2003 Oral changes associated with tobacco use. *Am J Med Sci* 326:179-182
36. **Kivlighan KT, Granger DA, Schwartz EB, Nelson V, Curran M, Shirtcliff EA** 2004 Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Horm Behav* 46:39-46

## Figure legends

Figure 1) Salivary cortisol levels (adjusted means including 95% confidence interval) over the day by smoking status,  $p=0.001$  in repeated measure analysis, adjusted for age, gender and last known employment grade, \*  $p<0.01$  for smokers compared to never smokers for individual sample.

Figure 2) Average cortisol release (adjusted means, error bars represent 95% confidence interval) by smoking status illustrated by the bar graph ANOVA  $p=0.008$ , adjusted for age, gender and last known employment grade, \*  $p=0.002$ .

Figure 3) Salivary cortisol levels (adjusted means including 95% confidence interval) over the day by numbers of cigarettes smoked and never smokers,  $p=0.646$  for dose response in repeated measure analysis,  $p<0.001$  compared to never smokers, adjusted for age, gender and last known grade.

Figure 4) Average cortisol release (adjusted means, error bars represent 95% confidence interval) by numbers of cigarettes smoked and never smokers illustrated by the bar graph, ANOVA  $p= 0.095$ , , adjusted for age, gender and last known grade, \*  $p<0.001$ .

Table 1) Participant characteristics for men and women who completed saliva sampling at phase 7 (2002-2004) of the Whitehall II study.

	Never	Ex	Smoker	
Percentage male	66.1	75.7	69.0	<0.001
Mean age (S.E.)	60.90 (60.77-61.03)	61.72 (61.58-61.86)	60.21 (59.93-60.49)	<0.001
<b>Social class</b>				
Percentage in the lowest grades	11.0	7.4	13.3	<0.001
Percentage in lowest income group	20.8	19.2	26.1	0.008
Percentage in lowest wealth group	7.3	6.3	15.5	<0.001
<b>Alcohol consumption</b>				
Percentage high weekly alcohol consumption *	13.9	24.8	29.2	<0.001
Percentage high on the sampling day alcohol consumption **	4.1	7.7	12.3	<0.001
<b>Physical characteristics</b>				
BMI >30	17.0	20.5	18.6	0.02
Percentage diabetic	4.5	4.7	5.1	0.809
Percentage having poor or very poor self rated health	12.9	15.4	21.9	<0.001
Percentage classed as depressed using GHQ assessment	11.2	9.7	14.4	0.02
<b>Stress</b>				
Percentage experiencing a stressful event on the day	6.8	7.1	7.8	0.748
Percentage stating more stress than usual on the day	14.3	12.5	13.4	0.268
Percentage financially insecure	10.6	8.4	15.4	<0.001

Numbers used for calculation of percentages ranges from 2817-3098 for men and 988-1184 for women due to missing information. ANOVA was used to assess any significant difference for age and chi squared test for all other variables. \* >21 units per week for men, >14 units per week for women. \*\*>4 alcoholic drinks on the day for men, >3 alcoholic drinks on the day for women

Table 2) Mean cortisol production over the day (nmol/lper hour) (95% C.I.) by smoking status adjusted for confounders and mediators

Smoking status	n	Adjustment for age, gender and employment grade	Plus adjustment for health behaviors *	Plus adjustment for psychological **
Never smokers	1676	4.13(4.02-4.24)	4.15 (4.04-4.26)	4.15 (4.04-4.26)
Ex-smokers	1267	4.21(4.08-4.35)	4.20 (4.07-4.33)	4.20 (4.07-4.33)
Smokers	323	4.67 (4.39-4.97)	4.62 (4.34-4.92)	4.63 (4.35-4.93)
ANOVA		0.002	0.008	0.008

\*alcohol consumption, BMI >30, poor self rated health

\*\*depression symptoms, financial insecurity







