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CLINICAL STUDY

Salivary cortisol in a middle-aged community sample: results from 990 men and women of the KORA-F3 Augsburg study

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Abstract

Objective: Analysis of salivary cortisol concentrations and derived indices is increasingly used in clinical and scientific medicine. However, comprehensive data on these parameters in the general population are scarce. The aim of this study was to evaluate the concentrations of salivary cortisol in a large middle-aged community sample and to identify major factors associated with altered hormone levels.
Design: We conducted a cross-sectional study within the Cooperative Health Research in the Region of Augsburg (KORA)-F3 study. A total of 1484 participants aged 50–69 years (52% women) had agreed to provide four saliva samples during a regular weekday.
Methods: We measured salivary cortisol concentrations at wake-up (F0), $^1/_2$ h ($F^1/_2$), 8 h (F8), and 14 h (F14) after waking. We calculated cortisol awakening response (CAR), slope, and area under the curve (AUC_G) of the circadian cortisol secretion. Sociodemographic and clinical characteristics were evaluated by interview and questionnaires, sampling conditions by protocol. In total, 1208 participants returned saliva samples, exclusion criteria left 990 subjects for final analyses.
Results: Salivary cortisol levels were (means$\pm$S.D.) F0$=13.7\pm7.6$, $F^1/_2=20.5\pm9.8$, F8$=5.4\pm3.3$, and F14$=2.0\pm1.8$ nmol/l. Earlier sampling times were associated with higher CAR and smaller slope. Cortisol secretion was also influenced by gender and smoking habits. Higher perceived social support was associated with lower AUC_G and smaller slope.
Conclusions: We provide data on salivary cortisol concentrations in a large middle-aged community sample. Gender, sampling time, smoking habits, and perceived social support appeared as determinants of cortisol secretion.

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Introduction

Salivary cortisol sampling is increasingly used for clinical and scientific purposes (1, 2). The concentration of this hormone in saliva parallels the free, biologically active plasma cortisol levels, and is independent of the rate of saliva production (3, 4). It gives information on the activity of the hypothalamus–pituitary–adrenal (HPA) system, a major player in the regulation of many body functions, such as glucose homeostasis and immune function. Measuring late-night salivary cortisol concentrations has been advocated as a screening test for Cushing's syndrome (5). Furthermore, altered cortisol levels have been described in common somatic and mental disorders. HPA system dysfunction, for example, is associated with major depression, and hypercortisolemia has been demonstrated in the majority of patients with an acute depressive episode (6). Altered cortisol levels have also been observed in diabetes mellitus (7) or arterial hypertension (8), but the significance of these findings is still unclear. In psychophysiology, salivary cortisol sampling is frequently used to unravel the body's response to different stressors (9), and altered cortisol secretion patterns have been observed in posttraumatic stress disorder, burn-out syndrome, work or marital stress, and other conditions (10).

Despite the wide dissemination of salivary cortisol sampling, there are only few studies providing comprehensive data on salivary cortisol concentrations in the general population (11–13). Existing information is limited, as it is derived from non-community cohorts (14, 15), or because it is focused on associations of saliva cortisol with selected

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variables (16–19). Accordingly, we chose to analyze salivary cortisol in a community sample, which was well characterized with regard to sociodemographic data, sampling factors, health habits, and both physical and mental health. As our interest focused on patterns of cortisol secretion associated with major somatic and mental disorders, we selected subjects aged 50–69 years, in whom the prevalence of many of these disorders is increased. Our aim was to meticulously describe the characteristics of salivary cortisol secretion and to unravel factors associated with altered hormone levels.

Methods

Study sample

Our study originates from the city of Augsburg (Bavaria, Germany) and surrounding districts, with ~600 000 inhabitants in urban and rural areas. The Cooperative Health Research in the Augsburg Region, Germany (KORA)-F3 study represents a follow-up study monitoring trends and determinants on cardiovascular diseases (MONICA)-S3 survey, a stratified random representative sample of 6481 eligible subjects that were drawn in 1994/1995 from the Augsburg population, of whom a total of 4856 subjects participated in the S3 baseline survey. At the time of the KORA-F3 follow-up study one decade later (2004/2005), a total of 405 (8%) subjects had died. Furthermore, subjects were considered ineligible for inclusion in the F3 follow-up survey if they lived too far outside the study region, were completely lost to follow-up ($n=222$, 5%), or had requested deletion of their address data ($n=270$, 6%). Of the remaining 3959 eligible subjects, 161 could not be contacted, 295 were unable to come because they were too ill, and 497 were not willing to participate, resulting in an interim total of 3006 participants in the F3 follow-up survey (response rate: 76% of S3 participants). As additional efforts were made to reach the 1300 eligible subjects from the original S3 sampling frame who had not participated in the S3 baseline survey, another 178 (14%) were included in the present KORA-F3 study, resulting in a total sample size of 3184 (overall response rate: 49.1%).

For our substudy, all 1515 participants aged 50–69 years were asked to provide salivary cortisol samples. All participants had given written informed consent, and the study had been approved by the local ethics committee. In all, 1484 study subjects consented to salivary collection, and 1208 (81.4% of consenting subjects) actually returned their saliva samples. We excluded 93 subjects due to documented incompliance with the sampling procedure, 67 subjects for exceeding the allowed 7-day time period between salivary collection and sample arrival (20), 23 due to systemic glucocorticoid therapy, and 12 as they had reached an age outside the defined range at the time of sampling. We also excluded samples with saliva cortisol concentrations exceeding three SDs above the mean and subjects who had woken up beyond the range of plus/minus three SDs of the mean wake-up time of 0744 h (0422–1106 h, five subjects).

Table 1 Characteristics of subjects with salivary cortisol samples ($n=990$). ± values represents SD.

	Value	*n* with valid data
General description		
Age (years)	60.2±5.5	990
Gender (%)		990
Male	48.0	
Female	52.0	
Marital status (%)		990
Married	82	
Single	5	
Divorced	6	
Widowed	7	
Education (years)	11.1±2.4	985
Occupational status (%)		
Working	34.8	990
Not working	65.2	
Sampling factors		
Sampling in a month with more daylight (%)	55.6	990
Wake-up time (h:min)	7:44±1:04	989
Health habits, anthropometry		
Physical activity (%)		990
Physically active	56.0	
Physically inactive	44.0	
Smoking status (%)		
Currently smoking	11.9	990
Former smoked	38.5	
Never smoked	49.6	
BMI (kg/m^2)	28.1±4.4	989
Physical health		
Lipid disorder (%)	35.3	990
Diabetes mellitus (%)	7.5	990
Arterial hypertension (%)	58.1	989
Ischemic heart disease (%) with history of	4.0	990
Myocardial infarction (%)	2.2	
ACVB (%)	1.4	
Angioplasty (%)	2.4	
Medication (%)		990
ACE inhibitor	12.3	
Antiplatelet agent	11.4	
β-Adrenergic inhibitor	19.4	
Lipid-lowering drug	12.6	
Hypoglycemic agent	6.3	
Mental health and psychosocial factors		
Subjective health (SF-12 score)		864
Physical dimension	47.4±8.8	
Mental dimension	51.8±9.0	
Depressive symptoms (PHQ-9 score)	3.4±3.5	988
Beliefs about determinants of health (HLOC score)		
Internal: self-responsibility	11.0±1.8	964
Social support (social support questionnaire score)	4.1±0.7	964

Salivary cortisol sampling

In the KORA study center, Augsburg, participants were individually instructed on the saliva sampling procedure by trained study personnel; additionally, detailed written information was provided. Subjects were asked to sample four saliva aliquots during the course of one day with the use of cotton-based sampling devices and to store them in plastic syringes (Salivette, Sarstedt, Nuembrecht, Germany). Absorbent cotton rolls were allowed to soak the saliva for 2 or 3 min, after which they were placed in syringes and kept in the participant's refrigerator. In case of acute illness, saliva collection was to be delayed until complete cessation of all symptoms. Samples were to be collected without delay upon waking (F0), followed by a salivette ½ h (F½), 8 h (F8), and 14 h (F14) after waking. Saliva sampling was to be scheduled on a week day with usual activities, but no specific time-point for wake-up was given. Subjects were instructed not to eat, drink, smoke, brush their teeth, or engage in physical activities for at least 30 min before saliva sampling. Sampling times and adherence to the correct sampling procedure had to be documented in a written protocol. Participants were asked to send salivettes and protocols to the Central Institute the day after sampling using the prepaid packaging provided. A reminder was sent to participants who had not returned samples after 2 weeks. Upon receipt, samples were frozen and stored at −80 °C. A time-resolved immunoassay with fluorescence detection was used for cortisol analysis in the laboratory of Clemens Kirschbaum, Dresden, Germany, with coefficients of intra- and interassay variation of $<8\%$.

Cortisol secretion indices

Activation of cortisol secretion associated with wake-up and starting the day (cortisol awakening response, CAR) was calculated by subtracting the F0 from the F½ value (21). Decline of cortisol secretion over the course of the day was expressed by the slope, calculated by subtracting evening F14 from wake-up F0 values and dividing the result by the number of hours separating both samples. In order to obtain an estimate of the total hormone output, the area under the curve with respect to ground (AUC_G) of F0, F8, and F14 was computed according to the trapezoidal method described by Prüssner (22). We chose not to include the F½ measure in slope and AUC_G calculation, as it has been suggested that this value, determining the CAR, is differentially regulated (23).

Characterization of participants

Each participant was subjected to a detailed interview, an anthropometric examination and a nonfasting blood sampling at the KORA study center; additional information was collected using standardized questionnaires. Sociodemographic data were collected during personal interviews. If the sampling date fell into the period from March 1 to August 31, it was assumed that sampling took place on a day with more daylight hours. Subjects were classified as active if they regularly participated in sports in summer and winter and if they were active for at least 1 h per week in either season (24). A standardized protocol was used to measure anthropometry, and participants' body mass index was calculated as weight in kilograms divided by height in square meters. A lipid disorder was assumed if subjects reported increased cholesterol or triglyceride levels during the past 12 months. Awareness of a diabetic disorder or ingestion of antiglycemic agents categorized subjects as diabetic, blood pressure values $\geq 140/90$ mmHg, or ingestion of antihypertensive medication, given that subjects were aware of being hypertensive, as hypertensive. An ischemic heart disease was assumed if participants reported a former myocardial infarction confirmed by a physician, a coronary artery bypass graft operation or a percutaneous coronary angioplasty with or without stent placement. Information on medication was ascertained by scanning the bar codes of subjects' medication. Questionnaires were used to asses mental health, with the SF-36 Health Survey Questionnaire, 12-item version (SF-12) (25) providing insight into subjective health, the Patient Health Questionnaire, 9-item version (26) assessing depressive symptoms, the Health Locus of Control-Subscale 'Self-responsibility' (27) addressing probands' beliefs about this factor's influence on their own health, and the Social Support Questionnaire, 14-item version (28) assessing social support.

Table 2 Saliva cortisol concentrations and cortisol secretion indices.

	n	**Time** (range) (h:min)	**Mean**	**S.D.**	**Median**	**Range**
Saliva cortisol concentration						
F0 (nmol/l)	888	7:44 (4:40–11:05)	13.7	7.6	12.4	0.3–59.7
F½ (nmol/l)	887	8:15 (5:10–14:34)	20.5	9.8	19.6	0.4–71.4
F8 (nmol/l)	901	15:55 (9:00–21:00)	5.4	3.3	4.7	0.1–19.8
F14 (nmol/l)	842	21:53 (18:40–1:05)	2.0	1.8	1.5	0.0–13.1
Cortisol secretion indices						
CAR (nmol/l)	718		7.4	11.2	6.8	−46.6–68.9
Slope (nmol/l per h)	751		0.83	0.53	0.76	−0.21–4.10
AUC_G (nmol/l per h)	702		100.7	43.5	93.2	8.7–303.5

Statistical analysis

Kendall's tau rank correlation coefficients were computed to test the degree of correspondence of cortisol measures. To determine the relative contribution of sociodemographic, sampling and health factors on cortisol secretion indices, separate linear regression analyses were run with CAR, slope, and AUC_G as dependent variables. For all analyses, models contained all covariates. In order to minimize inflation of α-error due to multiple testing, regression analyses were restricted to CAR, slope, and AUC_G. Tests were two-tailed, and a P value of ≤ 0.05 was considered to indicate statistical significance. Data were analyzed using SPSS software, version 15, for Windows (SPSS Inc., Chicago, IL, USA).

Results

Descriptive analyses

The study protocol gave clear instructions on time periods between salivary samples; however, not all subjects followed these objectives. Therefore, time windows were defined for acceptance or rejection of samples that had been collected outside the exact time periods. These time windows were 20–40 min for $F^1/_2$, 7–9 h for F8, and 13–15 h for F14 (22) respectively. Exclusion of 18 subjects with salivary sampling outside these windows left 990 subjects for final analyses. Their sociodemographic and clinical data are presented in Table 1.

Results of all four saliva samples were available in 819 of these subjects (82.7%). In 105 cases (10.6%), there were three, in 54 cases (5.5%), there were two, and in 12 cases (1.2%), there was one valid saliva cortisol sample available. Reasons for missing results were nonreturn of samples, too little saliva for analysis or sampling outside the defined time windows. Mean and median values, S.D., and range of saliva cortisol concentrations and cortisol secretion indices are given in Table 2, distribution of data in Fig. 1. Cortisol levels at $^1/_2$ h after awakening were 49.6% higher than awakening levels. Cortisol secretion indices appeared to roughly follow a normal distribution. The F0, $F^1/_2$, F8, and F14 values of men versus women, smokers versus nonsmokers, early versus late awakener, and subjects with lower versus higher social support are given in Fig. 2.

Correlational analyses

Salivary cortisol concentrations F0, $F^1/_2$, F8, and F14 were found to correlate only modestly (correlation coefficients: 0.07–0.24, Table 3), with F8 and F14 showing the highest coefficient ($\tau = 0.24$). Correlations of F0, $F^1/_2$, F8, and F14 with the cortisol secretion indices CAR, slope, and AUC_G were markedly influenced by the arithmetical methods of generating these indices, e.g. AUC_G highly correlated with F0 ($\tau = 0.61$), simply because F0 constitutes the value that most strongly contributes to AUC_G calculation. However, some findings shall be highlighted: CAR was found to negatively correlate with $F^1/_2$ ($\tau = -0.30$), and showed no correlation with F14 ($\tau = -0.01$). Also the slope was strongly correlated with F0 ($\tau = 0.86$), but not with F14 ($\tau = 0.03$). CAR correlated only weakly (negatively) with AUC_G ($\tau = -0.15$) and moderately (negatively) with the slope ($\tau = -0.32$). Correlation between indices was highest for AUC_G and slope ($\tau = 0.53$).

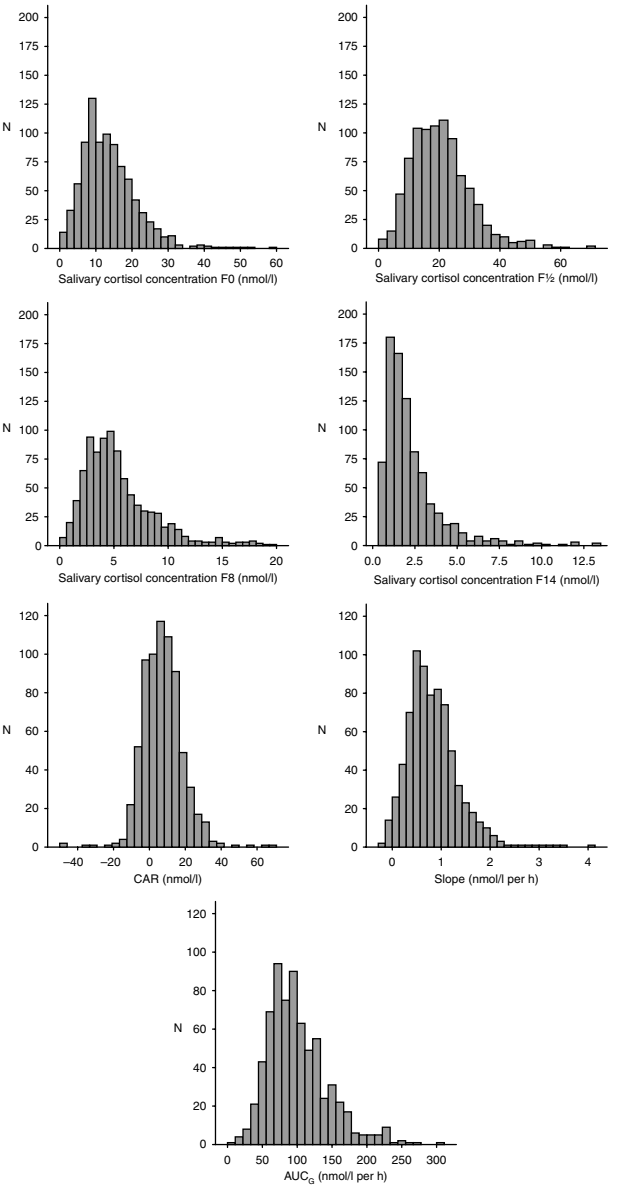

Figure 1 Saliva cortisol concentrations F0, $F^1/_2$, F8, F14, and CAR, slope, and AUC_G.

Regression analyses

As expected, CAR was strongly influenced by the time of awakening (F0 sampling time), with earlier wake-up times being associated with a greater CAR (Table 4). Wake-up time also altered the slope, with later wake-up being associated with a greater (steeper) slope. Slope was also influenced by smoking habits and extent of reported social support; both nonsmoking status and higher perceived social support were associated with a smaller slope. AUC_G was higher in men and in subjects reporting less social support. Interestingly, AUC_G was not associated with F0 sampling time.

Discussion

The large size of our sample provided the opportunity to diligently characterize cortisol secretion in community-dwelling subjects. Saliva cortisol levels during the day were found to be in line with those observed in other large samples (14, 16). The distribution of cortisol concentrations (Fig. 1) displays a large interindividual variability. This finding reflects the complexity of cortisol regulation, which includes a wide array of internal and external factors. Differences in glucocorticoid receptor sensitivity due to common genetic polymorphisms may constitute one of these internal determinants (29). Daily social and emotional experiences are examples for external factors (11, 12). Salivary cortisol concentrations also show a marked skewing with a right hand tail, which is more clearly visible in F8 and F14 samples. Also, greater proportions of F8 and F14 samples exceeded the mean by three SDs, and were excluded as outliers. This finding may be caused by additional factors in a subset of the sample, which are more influential in the afternoon and evening. Owing to the periodic circadian system, cortisol secretion activity slows down during this period, and other factors gain influence. Endocrine disorders (e.g. Cushing's syndrome) may constitute one of these factors. Epidemiologic studies suggest an incidence of Cushing's syndrome ranging from 0.7 to 2.4 per million population per year and a prevalence of 2–5% in obese patients with poorly controlled type 2 diabetes and hypertension (30). Clearly, this factor has to be considered in studies on salivary cortisol, especially when afternoon and evening samples are examined. Another interesting finding is the substantial proportion (25.3%) of subjects with negative CAR values. In these individuals, salivary cortisol concentrations upon awakening exceeds that of 30 min after getting up. As CAR data display a normal distribution, external factors, e.g. incompliance with the sampling procedure (31), seem insufficient as sole explanation. It appears possible that a significant proportion of these 'CAR nonresponders' consists of subjects with poor sleep quality and frequent awakening during the course of the night, in whom the CAR was partially or completely missed with our sampling procedure (32). However, one might also assume that a negative CAR is part of normal cortisol regulation in a subset of middle-aged community-dwelling subjects.

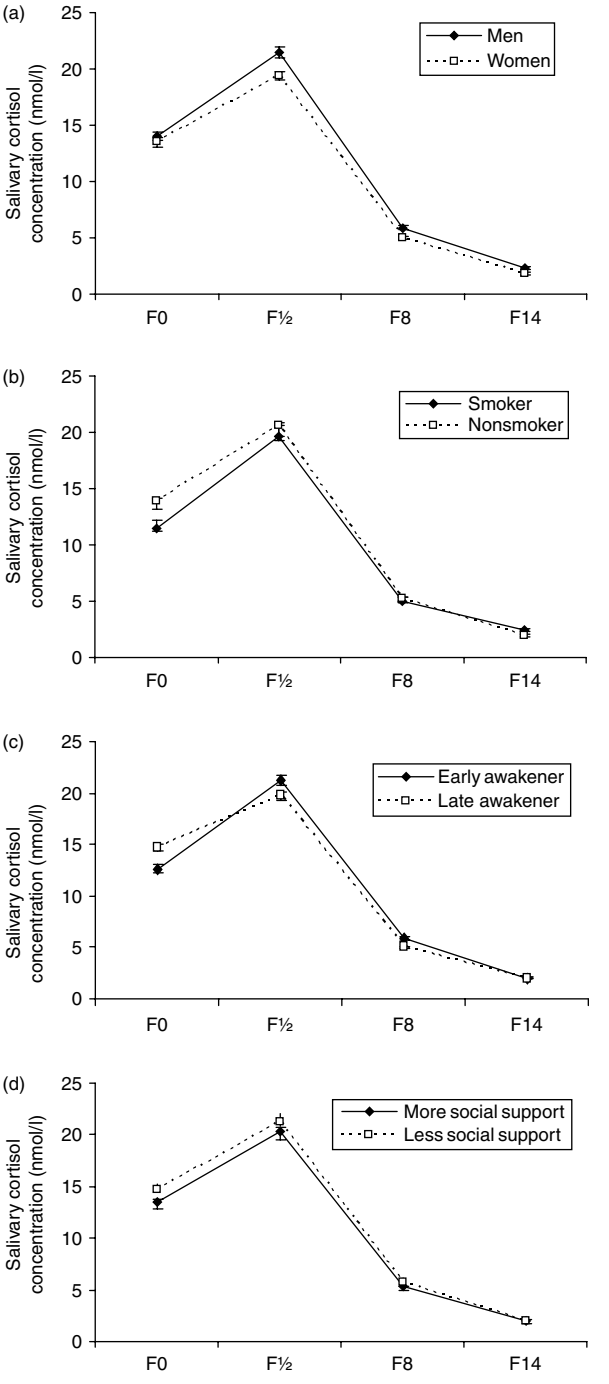

Figure 2 Saliva cortisol concentrations in (a) men versus women, (b) smoker versus nonsmoker, (c) early versus late awakener, and (d) subjects with more versus less social support. Error bars denote S.E.M.

Salivary cortisol concentrations during the course of a day and cortisol secretion indices were found to be at most moderately intercorrelated. This finding reflects the great extent of internal and external factors determining saliva cortisol concentrations in the individual. The low association of afternoon and evening values with the secretion indices, CAR and slope, support the assumption of a different regulation of these measures.

As expected (33–36), individuals with earlier wake-up time showed a higher CAR, possibly because lower F0 concentrations early in the morning allowed for a greater hormone rise. Furthermore, a ceiling effect may exist when F0 values are elevated during the late-morning hours. Wake-up time also influenced slope, which was smaller in subjects who woke up earlier. This association may also by explained by the finding mentioned above, as the slope is greatly determined by the F0 values, which are lower in early awakeners. If circadian cortisol secretion was summarized as AUC_G, men showed higher hormone values as compared with women, a finding in line with other studies (37). Furthermore, smoking habits were associated with the steepness of the slope with nonsmokers displaying a smaller decrease in cortisol secretion over the day. Smoking is known to have a consistent effect on cortisol secretion measures (14).

Among the psychosocial and mental health factors included in the analyses, we found an association of perceived social support and cortisol secretion. Higher social support, as reported by the study participants, was associated with lower AUC_G and smaller slope. This finding extends observations from other researchers. During an experimental stress test, the rise in cortisol secretion was greatly diminished, when a close friend provided social support during the preparation period (38). In the Whitehall II cohort, individuals who coped by problem engagement and by seeking social support had lower cortisol levels (37). Our report is the first to provide evidence that this psychological factor is independently linked with cortisol secretion in a community sample.

Another prominent finding of our study is the absence of an association between depressive symptoms and cortisol secretion indices. Whereas elevated cortisol levels have repeatedly been demonstrated in hospitalized patients with a major depressive episode (39, 40), studies of depressive subjects outside the hospital gave mixed results, with either increased (41) or normal (42, 43) hormone levels. Also, subjects with diabetes, coronary artery disease or arterial hypertension did not differ in the cortisol secretion indices used in our analysis. It was postulated that HPA system dysfunction should predispose individuals to the formation of these ailments, possibly by untoward effects on cardiovascular or endocrine regulation (44). However, other cortisol variables like single measures may vary between groups. For example, F14 evening cortisol levels were higher in subjects with diabetes as

Table 3 Correlation of salivary cortisol concentrations and cortisol secretion indices.

	F0	F½	F8	F14	CAR	Slope	AUC_G
F0							
Correlation coefficient	1.0	0.18	0.10	0.11	−0.30	0.86	0.61
Significance		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
n	888	824	820	762	715	751	702
F½							
Correlation coefficient		1.0	0.21	0.07	0.51	0.17	0.27
Significance			<0.001	<0.01	<0.001	<0.001	<0.001
n		887	815	768	717	708	668
F8							
Correlation coefficient			1.0	0.24	0.13	0.07	0.48
Significance				<0.001	<0.001	<0.01	<0.001
n			901	776	678	703	702
F14							
Correlation coefficient				1.0	−0.01	−0.03	0.27
Significance					0.63	0.24	<0.001
n				842	624	751	702
CAR							
Correlation coefficient					1.0	−0.32	−0.16
Significance						<0.001	<0.001
n					718	614	584
Slope							
Correlation coefficient						1.0	0.53
Significance						.	<0.001
n						751	702
AUC_G							
Correlation coefficient							1.0
Significance							
n							702

Correlaton coefficients are Kendall's tau b (τ).

Table 4 Multivariable analyses of study subjects' characteristics and cortisol secretion indices (standardized regression coefficients and *P* values).

	CAR		Slope		AUC_G	
	β	*P*	β	*P*	β	*P*
General description						
Age (years)	−0.09	0.11	−0.04	0.44	−0.03	0.56
Gender (0=male, 1=female)	−0.02	0.67	−0.03	0.60	−0.13	**0.006**
Marital status: married (0=no, 1=yes)	0.00	0.99	0.00	0.92	−0.03	0.50
Education (years)	0.02	0.73	0.00	0.94	0.04	0.40
Working (0=no, 1=yes)	0.01	0.89	−0.06	0.29	−0.09	0.15
Sampling factors						
More daylight (0=no, 1=yes)	0.06	0.18	0.04	0.41	0.08	0.08
Wake-up time (h:min)	−0.21	<**0.001**	0.15	**0.001**	0.01	0.92
Health habits, anthropometry						
Physically active (0=no, 1=yes)	−0.03	0.47	0.00	0.90	−0.03	0.48
Smoking (0=no, 1=yes)	−0.05	0.25	0.15	**0.001**	0.04	0.35
BMI (kg/m^2)	0.03	0.48	−0.05	0.23	−0.07	0.16
Physical health						
Lipid disorder (0=no, 1=yes)	0.01	0.78	0.03	0.47	−0.02	0.69
Diabetes mellitus (0=no, 1=yes)	−0.04	0.34	0.01	0.79	0.03	0.49
Arterial hypertension (0=no, 1=yes)	−0.03	0.53	−0.04	0.38	0.01	0.78
Ischemic heart disease (0=no, 1=yes)	−0.03	0.56	0.01	0.86	0.04	0.43
Mental health and psychosocial factors						
Subjective health, physical dimension (SF-12 subscore)	0.02	0.63	0.00	0.97	−0.01	0.87
Subjective health, mental dimension (SF-12 subscore)	0.08	0.16	0.04	0.53	0.00	0.94
Depressive symptoms (PHQ-9 score)	0.06	0.31	−0.03	0.64	−0.04	0.52
Belief about self-responsibility as determinant of health (HLOC subscore)	0.03	0.44	−0.01	0.77	−0.01	0.76
Social support (social support questionnaire score)	−0.05	0.25	−0.09	**0.05**	−1.0	**0.03**

Significant *P* values are highlighted in bold face

compared with nondiabetic participants (2.5 ± 2.1 vs 2.0 ± 1.8 nmol/l, Mann–Whitney *U* test, $P=0.04$), in line with findings of Liu *et al.* (45), although multivariable analysis revealed that diabetes status did not contribute to explanation of F14 variance ($\beta=0.04$, $P=0.35$). Hypothetically, this finding could be due to low case number (diabetic subjects, $n=66$) or variability of F14 sampling time (range, 18:40–1:05 h). Also, the lacking association of age with any of our cortisol indices should be viewed with caution. Elevated evening cortisol levels have been observed in elderly subjects, as compared with younger controls (46). Potentially, our age range of 50–69 years was too small to allow for the detection of such an association.

Some strengths and limitations of our study should be noted. Our study sample was large and well characterized, providing a precise in-depth view of results. Owing to the age stratum, cortisol levels in women were unaffected by menstrual cycle or oral contraception. Also, our sample was not distorted by exclusion of any subgroups and thus gave a precise view of a community-dwelling population. However, we cannot exclude a bias in our data due to the subjects who did not participate in KORA-F3, e.g. because they were too ill (295 subjects). A major limitation inherent to home-based saliva sampling is potential error in sampling time and procedure as well as in the order of the samples. Also, we did not use electronic devices to check for adherence to the sampling procedure. However, personal instruction of each participant, written information, request for a precise protocol, and exclusion of subjects reporting incorrect sampling should have supported correct results. Another potential limitation was the range of F0 sampling, which may have interfered with the circadian HPA activity and may have blurred existing associations of cortisol secretion with other factors. However, we chose not to interfere with subjects' daily routines, in order to obtain a naturalistic profile determined by the individuals' regular activities. Finally, data on illness prevalence were based on self-report, and might have missed undiagnosed cases.

In conclusion, we present data on salivary cortisol concentrations in a large middle-aged community sample. Major determinants of cortisol secretion include gender, sampling time, smoking habits, and perceived social support.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

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References

1 Laudat MH, Cerdas S, Fournier C, Guiban D, Guilhaume B & Luton JP. Salivary cortisol measurement: a practical approach to assess pituitary–adrenal function. *Journal of Clinical Endocrinology and Metabolism* 1988 **66** 343–348.
2 Kirschbaum C & Hellhammer DH. Salivary cortisol in psychobiological research: an overview. *Neuropsychobiology* 1989 **22** 150–169.
3 Bolufer P, Gandia A, Rodriguez A & Antonio P. Salivary corticosteroids in the study of adrenal function. *Clinica Chimica Acta* 1989 **183** 217–225.
4 Read GF, Walker RF, Wilson DW & Griffiths K. Steroid analysis in saliva for the assessment of endocrine function. *Annals of the New York Academy of Sciences* 1990 **595** 260–274.
5 Raff H, Raff JL & Findling JW. Late-night salivary cortisol as a screening test for Cushing's syndrome. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 2681–2686.
6 Pariante CM & Lightman SL. The HPA axis in major depression: classical theories and new developments. *Trends in Neurosciences* 2008 **31** 464–468.
7 Chiodini I, Adda G, Scillitani A, Coletti F, Morelli V, Di Lembo S, Epaminonda P, Masserini B, Beck-Peccoz P, Orsi E, Ambrosi B & Arosio M. Cortisol secretion in patients with type 2 diabetes: relationship with chronic complications. *Diabetes Care* 2007 **30** 83–88.
8 Wirtz PH, von Kanel R, Emini L, Ruedisueli K, Groessbauer S, Maercker A & Ehlert U. Evidence for altered hypothalamus–pituitary–adrenal axis functioning in systemic hypertension: blunted cortisol response to awakening and lower negative feedback sensitivity. *Psychoneuroendocrinology* 2007 **32** 430–436.
9 Hellhammer DH, Wust S & Kudielka BM. Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology* 2009 **34** 163–171.
10 Chrousos GP. Stress and disorders of the stress system. *Nature Reviews. Endocrinology* 2009 **5** 374–381.
11 Adam EK, Hawkley LC, Kudielka BM & Cacioppo JT. Day-to-day dynamics of experience–cortisol associations in a population-based sample of older adults. *PNAS* 2006 **103** 17058–17063.
12 Sjogren E, Leanderson P & Kristenson M. Diurnal saliva cortisol levels and relations to psychosocial factors in a population sample of middle-aged Swedish men and women. *International Journal of Behavioral Medicine* 2006 **13** 193–200.
13 Almeida DM, McGonagle K & King H. Assessing daily stress processes in social surveys by combining stressor exposure and salivary cortisol. *Biodemography and Social Biology* 2009 **55** 219–237.
14 Vreeburg SA, Kruijtzer BP, van Pelt J, van Dyck R, DeRijk RH, Hoogendijk WJ, Smit JH, Zitman FG & Penninx BW. Associations between sociodemographic, sampling and health factors and various salivary cortisol indicators in a large sample without psychopathology. *Psychoneuroendocrinology* 2009 **34** 1109–1120.
15 Kumari M, Badrick E, Sacker A, Kirschbaum C, Marmot M & Chandola T. Identifying patterns in cortisol secretion in an older population. Findings from the Whitehall II study. *Psychoneuroendocrinology* 2010 **35** 1091–1099. DOI:10.1016/j.psyneuen.2010.01.010.
16 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.
17 Dekker MJ, Koper JW, van Aken MO, Pols HA, Hofman A, de Jong FH, Kirschbaum C, Witteman JC, Lamberts SW & Tiemeier H. Salivary cortisol is related to atherosclerosis of carotid arteries. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 3741–3747.
18 Ranjit N, Diez-Roux AV, Sanchez B, Seeman T, Shea S, Shrager S & Watson K. Association of salivary cortisol circadian pattern with cynical hostility: multi-ethnic study of atherosclerosis. *Psychosomatic Medicine* 2009 **71** 748–755.
19 Thomas C, Hertzman C & Power C. Night work, long working hours, psychosocial work stress and cortisol secretion in mid-life: evidence from a British birth cohort. *Occupational and Environmental Medicine* 2009 **66** 824–831.
20 Clements AD & Parker CR. The relationship between salivary cortisol concentrations in frozen versus mailed samples. *Psychoneuroendocrinology* 1998 **23** 613–616.
21 Clow A, Thorn L, Evans P & Hucklebridge F. The awakening cortisol response: methodological issues and significance. *Stress* 2004 **7** 29–37.
22 Pruessner JC, Kirschbaum C, Meinlschmid G & Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 2003 **28** 916–931.
23 Wust S, Federenko I, Hellhammer DH & Kirschbaum C. Genetic factors, perceived chronic stress, and the free cortisol response to awakening. *Psychoneuroendocrinology* 2000 **25** 707–720.
24 Meisinger C, Kandler U & Ladwig KH. Living alone is associated with an increased risk of type 2 diabetes mellitus in men but not women from the general population: the MONICA/KORA Augsburg Cohort Study. *Psychosomatic Medicine* 2009 **71** 784–788.
25 Bullinger M & Kirchberger I. *SF-36 Fragebogen zum Gesundheitszustand*. Göttingen: Hogrefe, 1998.
26 Spitzer RL, Kroenke K & Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary care evaluation of mental disorders. Patient health questionnaire. *Journal of the American Medical Association* 1999 **282** 1737–1744.
27 Wallston KA, Wallston BS & DeVellis R. Development of the multidimensional health locus of control (MHLC) scales. *Health Education Monographs* 1978 **6** 160–170.
28 Fydrich T, Sommer G & Brähler E. *F-SozU: Fragebogen zur sozialen Unterstützung*. Göttingen: Hogrefe, 2007.
29 Huizenga NA, Koper JW, De Lange P, Pols HA, Stolk RP, Burger H, Grobbee DE, Brinkmann AO, De Jong FH & Lamberts SW. A polymorphism in the glucocorticoid receptor gene may be associated with and increased sensitivity to glucocorticoids *in vivo*. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 144–151.
30 Newell-Price J, Bertagna X, Grossman AB & Nieman LK. Cushing's syndrome. *Lancet* 2006 **367** 1605–1617.
31 Kudielka BM, Broderick JE & Kirschbaum C. Compliance with saliva sampling protocols: electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic Medicine* 2003 **65** 313–319.
32 Wilhelm I, Born J, Kudielka BM, Schlotz W & Wust S. Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology* 2007 **32** 358–366.
33 Edwards S, Evans P, Hucklebridge F & Clow A. Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology* 2001 **26** 613–622.
34 Kudielka BM & Kirschbaum C. Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology* 2003 **28** 35–47.
35 Federenko I, Wust S, Hellhammer DH, Dechoux R, Kumsta R & Kirschbaum C. Free cortisol awakening responses are influenced by awakening time. *Psychoneuroendocrinology* 2004 **29** 174–184.
36 Williams E, Magid K & Steptoe A. The impact of time of waking and concurrent subjective stress on the cortisol response to awakening. *Psychoneuroendocrinology* 2005 **30** 139–148.

37 O'Donnell K, Badrick E, Kumari M & Steptoe A. Psychological coping styles and cortisol over the day in healthy older adults. *Psychoneuroendocrinology* 2008 **33** 601–611.
38 Heinrichs M, Baumgartner T, Kirschbaum C & Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. *Biological Psychiatry* 2003 **54** 1389–1398.
39 Gibbons JL & McHugh P. Plasma cortisol in depressive illness. *Journal of Psychiatric Research* 1962 **1** 162–171.
40 Deuschle M, Schweiger U, Weber B, Gotthardt U, Korner A, Schmider J, Standhardt H, Lammers CH & Heuser I. Diurnal activity and pulsatility of the hypothalamus–pituitary–adrenal system in male depressed patients and healthy controls. *Journal of Clinical Endocrinology and Metabolism* 1997 **82** 234–238.
41 Vreeburg SA, Hoogendijk WJ, van Pelt J, Derijk RH, Verhagen JC, van Dyck R, Smit JH, Zitman FG & Penninx BW. Major depressive disorder and hypothalamic–pituitary–adrenal axis activity: results from a large cohort study. *Archives of General Psychiatry* 2009 **66** 617–626.
42 Posener JA, DeBattista C, Williams GH, ChmuraKraemer H, Kalehzan BM & Schatzberg AF. 24-Hour monitoring of cortisol and corticotropin secretion in psychotic and nonpsychotic major depression. *Archives of General Psychiatry* 2000 **57** 755–760.
43 Peeters F, Nicolson NA & Berkhof J. Levels and variability of daily life cortisol secretion in major depression. *Psychiatry Research* 2004 **126** 1–13.
44 Chrousos GP & Gold PW. A healthy body in a healthy mind – and vice versa – the damaging power of "uncontrollable" stress. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1842–1845.
45 Liu H, Bravata DM, Cabaccan J, Raff H & Ryzen E. Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans. *Clinical Endocrinology* 2005 **63** 642–649.
46 Raff H, Raff JL, Duthie EH, Wilson CR, Sasse EA, Rudman I & Mattson D. Elevated salivary cortisol in the evening in healthy elderly men and women: correlation with bone mineral density. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* 1999 **54** M479–M483.

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