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Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

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Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

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Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

ABSTRACT

Objectives: We conducted individual participant meta-analysis to test the hypothesis that cortisol patterns indicative of dysregulated HPA-axis functioning would be prospectively associated with poorer wellbeing at follow-up.

Setting: Four large UK-based cohort studies

Participants: Those providing valid salivary or serum cortisol samples (n=7515 for morning cortisol; n=1756 for evening cortisol) at baseline (age 44-82) and wellbeing data on the Warwick Edinburgh Mental Wellbeing Scale at follow-up (0-8 years) were included.

Results: Wellbeing was not associated with morning cortisol or diurnal slope though a borderline association with evening cortisol was found. Adjusting for sex and follow-up time, each 1 standard deviation increase in evening cortisol was associated with a -0.47 (95% CI -1.00, 0.05) point lower WEMWBS. This was attenuated by adjustment for body mass index, smoking and socioeconomic position. Between-study heterogeneity was low.

Conclusions: This study does not support the hypothesis that diurnal cortisol is prospectively associated with wellbeing up to 8 years later. However, replication in prospective studies with cortisol samples over multiple days is required.

Keywords: meta-analysis; individual participant data; positive psychology;

Strengths

- Individual participant meta-analysis based on 1612-7515 participants with cortisol

  data

- Prospective study with up to 8 years of follow-up

- Validated mental wellbeing instrument based on 14 items capturing hedonic and

  eudaimonic components harmonized across cohorts

Limitations

- Salivary cortisol samples taken over 1 or 2 days; up to a maximum of 4 times per day

- Actual waking time not recorded in all studies

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INTRODUCTION

Cortisol is a marker of hypothalamic-pituitary-adrenal (HPA) axis functioning and follows a diurnal rhythm. Large-scale epidemiological studies have measured salivary cortisol sampled several times during the course of a day to capture its rising levels during the awakening response and the subsequent decline across the day. The mental health and wellbeing consequences of raised cortisol levels are of interest particularly among older people given that they have higher evening cortisol levels[1,2] and greater total cortisol output throughout the day[3] compared with younger people.

Positive mental wellbeing is a multidimensional concept that captures hedonic (e.g. happiness and pain avoidance) and eudemonic (e.g. self-realization and psychologically functioning at potential) aspects of mental health that are distinct from depressive illness.[4,5] Cortisol may be linked to a positive psychological state through its effect on mood-altering neurotransmitters including serotonin.[6] Cortisol also has energy-mobilising properties that may in turn promote mental wellbeing.[7]

Two studies of healthy older people found no association between cortisol levels and positive wellbeing (captured by the positive items of the General Health Questionnaire[8] or by daily positive emotions[9]). A study of women aged 65 and over found that greater eudaimonic wellbeing was associated with flatter cortisol slope over the day, though this was due to lower morning cortisol levels which remained low across the day.[10] All studies were small (less than 200 participants). In younger adults (i.e. 55 years and younger), some studies have found that greater positive affect or happiness was associated with lower total cortisol output[11-14] but others have not.[15,16] These studies have not examined prospective

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associations between baseline cortisol patterns and wellbeing at follow-up in large,

population-based samples.

The aim of the current study was to draw on individual participant data from four

large British cohort studies using meta-analysis to examine the longitudinal associations

between diurnal cortisol and positive mental wellbeing captured by an instrument which

summarizes positive thoughts and feelings from both hedonic and eudemonic perspectives.

We hypothesized that cortisol patterns indicative of disrupted HPA axis functioning (i.e. lower

early morning levels, higher evening levels and less steep decline (i.e. flatter response) across

the day) would be associated with lower wellbeing at follow-up.

METHODS

Cohort studies

We used the four cohort studies from the Healthy Ageing across the Life Course (HALCyon)

cross cohort research programme[17] with data on both cortisol and Warwick Edinburgh

Mental Wellbeing Scale: the Caerphilly Prospective Study (CaPS)[18]; the Hertfordshire Cohort

Study (HCS)[19]; the MRC National Survey of Health and Development (NSHD)[20]; and the

National Child Development Study (NCDS).[21]

Caerphilly Prospective Study (CaPS)

CaPS is a cohort of men who were recruited when they were aged 45–59 years, between 1979

and 1983, from the town of Caerphilly and adjacent villages in South Wales. In the second

wave (1984 – 1988) the original cohort was supplemented with men of a similar age who had

moved into the defined area. Salivary cortisol was assessed at phase 5 of data collection in

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2000–2004 when participants were aged 65 to 82 years and wellbeing was assessed in 2011 when they were aged 73 to 90 years.

Hertfordshire Cohort Study (HCS)

HCS is a cohort of men and women born in East, North or West Hertfordshire between 1931 and 1939 whose birth and infant records were available and who were alive and still living in Hertfordshire in the 1990s. Cortisol was assessed at wave 1 of the data collection (1999–2004) when participants were aged 60 – 73 years and wellbeing was assessed in 2008 when they were aged 69 – 78.

The MRC National Survey of Health and Development (NSHD)

NSHD is a nationally representative sample of people born in England, Scotland, and Wales during one week in March 1946 followed prospectively since birth. Cortisol and wellbeing data were collected in 2008-10 when cohort members were aged 62-64 years.

The National Child Development Study (NCDS)

NCDS is a nationally representative sample of people born in England, Scotland, and Wales during 1 week in March 1958 followed prospectively since birth. Cortisol was assessed as part of a bio-medical survey (2002–2004) when cohort members were aged 44-45 and wellbeing was assessed at a follow-up (2008-2009) at mean age of 50.7.

Cortisol

In HCS, just one fasting morning serum cortisol sample was ascertained from each participant, frozen and subsequently measured by radioimmunoassay. Salivary cortisol samples were

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collected in CaPS, NSHD and NCDS at multiple times across the day with participants shown

how to collect saliva using plain cotton wool swabs (salivettes) at home. Subjects were asked

to chew on the salivettes for 1-2 min and a saliva sample was obtained. In CaPS, participants

were requested to take samples on waking, 30 minutes later, at 2 pm and 10 pm on two

consecutive days. In NSHD, samples were taken on waking, 30 minutes later and at 9 pm.

Samples in NCDS were taken in the first 45 minutes after waking and 3 hours later. Samples

from CaPS, NSHD and NCDS were frozen and subsequently assayed by radioimmunoassay

done at the University of Dresden which specializes in high through-put cortisol assays.[22]

Mental wellbeing

Positive mental wellbeing was assessed using the Warwick Edinburgh Mental Wellbeing Scale

(WEMWBS) in all four studies. This self-completion scale captures positive affect, satisfying

interpersonal relationships and positive functioning. Items are worded positively and

respondents are asked to indicate how frequently, on a five point scale, they have

experienced each statement over the last two weeks. Statements include "I've been feeling

good about myself", "I've been feeling close to other people", "I've been interested in new

things" and "I've been feeling optimistic about the future". Scores theoretically range from 14

to 70 with 70 indicating highest wellbeing. Where three or fewer items were missing, values

were imputed based on the average score for completed items (CaPS n=43; HCS n=52; NSHD

n=84; NCDS n=103). Internal consistency of the scale in all four cohorts was high (Cronbach

alpha=0.91 in HCS, NSHD and NCDS and 0.93 in CaPS.[23] Validation work indicates good

construct validity for a single factor structure as well as good criterion validity and test-retest

reliability and supports its use in general population samples.[24]

Covariates

Key covariates that might confound the association between cortisol and wellbeing and which

had been assessed in each of the cohorts were chosen *a priori*: sex, age at cortisol

measurement, follow-up time to measurement of wellbeing, body mass index (BMI), smoking,

and adult socio-economic position.[25-27] The covariates were measured at the same wave

as cortisol samples unless otherwise stated. BMI was calculated as measured weight divided

by the square of height and was categorized into quartiles when there was evidence of

deviation from linearity in the association with wellbeing (NSHD, NCDS). Smoking was

reported by participants and dichotomized into current smoker vs ex- and never smoker

(NCDS smoking data at age 42 years). Adult socioeconomic position was derived from own

occupational class (CaPS at age 47-67, HCS at age 60-73, NSHD at age 53 or earlier if missing,

NCDS at age 42) and grouped as manual or non-manual occupation (the latter indicating

greater socioeconomic advantage).

Initial treatment of the data and standardization

Cortisol has a marked circadian rhythm and therefore the time of day at which it is sampled

will affect its level. In CaPS, NSHD and NCDS actual times when the salivary samples were

taken were recorded by participants. Observed values were adjusted for the time of sampling

by fitting a linear or polynomial function to the association between cortisol and time of

measurement and adding the resulting residuals from the best fit model to the overall mean

cortisol value. This gives the estimated cortisol level at the time specified in the protocol for

each participant. Morning cortisol (salivary or serum) levels were available in all four cohorts

(CaPS, HCS, NSHD and NCDS) and night time values in CaPS and NSHD. For NCDS, the later

cortisol measure was taken 3 hours after the morning measure on the same day (around

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11.15AM) and there was no evening measure. However past publications support the notion that the diurnal decline over this shorter period is a good surrogate for the decline from morning till night[28] and hence this measure was used to derive a measure of diurnal slope. HCS collected serum cortisol, the levels of which are around 20 times higher than the free cortisol concentrations found in saliva. However, a study on the relationship between serum and salivary cortisol in healthy individuals[29] showed that correlations were high whether taken at the same time (>0.90) or 70 minutes apart (0.54—0.94). Those participants who reported taking cortico-steroid medication were excluded from the analysis sample (CaPS n=62; HCS n=13; NSHD n=19). In NCDS, participants were excluded from analysis if they reported taking endocrine system medication (n= 396). Salivary cortisol values greater than 100 nmol/L were removed (CaPS n=5; NSHD n=7; NCDS n=21), since high cortisol values can have substantial statistical influence on estimates and it is unclear what these high values represent.[30] Morning salivary cortisol values that were not between 5am and noon were removed and evening values if they were before 8pm, since these participants may be shift-workers with substantially different cortisol profiles.[31] In CaPS, cortisol values were averaged over the same measures obtained on two consecutive days. Early morning salivary cortisol was computed in CaPS and NSHD as the mean of waking and 30 min samples; to be comparable, in NCDS the cortisol measure taken within 45 minutes after waking was used. In HCS morning serum cortisol was used.

To be able to combine the cohorts in a meta-analysis, the cortisol values were standardized by deriving study-specific z-scores. In NCDS, both early and late morning cortisol were positively skewed, as was night time cortisol in CaPS and NSHD, so values were $\log_e$ transformed before they were converted to z-scores. In addition to the early morning and

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night time cortisol measures, we derived the diurnal slope (CaPS, NSHD, NCDS) as early morning value subtracted from the evening (or late morning in NCDS) value and divided by the lapsed time period. The overall slope is negative and so positive z-scores indicate a flatter response.

Statistical Methods

A two stage meta-analysis was performed. In the first stage, we modelled WEMWBS as a function of each diurnal cortisol indicator in turn using linear regression in each cohort separately, with adjustment for i) sex, age at cortisol measurement and follow-up time to the wellbeing measurement, ii) additional adjustment for BMI, smoking status and socioeconomic position. There was no evidence of deviation from linearity in the association between any of the cortisol measures and WEMWBS. There was no evidence of interaction between sex and any of the cortisol measures. In sensitivity analysis to explore possible bias arising from missing covariates , we adjusted for sex and age at cortisol and follow-up time i) using the maximum available sample with cortisol and wellbeing data (results not presented), ii) using the sample restricted to only those participants that had complete data on all covariates. Results did not materially differ for these two samples. In the second stage, cohort-specific estimates were pooled in random-effects meta-analyses[32] chosen a priori due to the expected heterogeneity between the different studies.

Sensitivity analyses

We corrected regression estimates for regression dilution bias arising from error in the measurement of cortisol. The reliability ratios were estimated by regressing the cortisol

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measure on day 2 on the measure on day 1 from CaPS data.[33] This yielded reliability ratios

of 0.554, 0.349 and 0.430 for morning, evening and slope cortisol respectively.

RESULTS

The characteristics of the participants of the four cohorts in the analysis are shown in Table 1.

Age at time of wellbeing measurement ranged from mean (sd) age of 50.7 (0.1) years in NCDS

to 80.1 (3.9) years in CaPS. Mean wellbeing ranged from 49.5 (7.9) in NCDS to 53.3 (10.6) in

CaPS and increased with mean age of the cohort. Mean early morning cortisol values were

similar for the three cohorts (CaPS, NSHD, NCDS) that had measured salivary cortisol.

We found no evidence of an association between early morning cortisol and wellbeing in the

individual cohorts in sex, age and follow-up time adjusted models. The overall pooled estimate

was 0.02 (95% CI -0.17,0.21; p=0.8) and there was no evidence of heterogeneity across studies

(I^2=2.3; p for heterogeneity=0.4) (Table 2 and Figure 1A). Further adjustment for all covariates

did not affect the overall pooled estimate 0.01 (95% CI -0.22,0.24; I^2=18.0; p for

heterogeneity=0.3).

Sex, age and follow-up time adjusted associations between evening cortisol and wellbeing in

the individual cohorts were in the expected direction (i.e. higher evening cortisol was

associated with lower wellbeing) in NSHD:-0.33 (95% CI -0.77,0.11); CaPS -0.98 (95% CI -

2.03,0.07). This indicates a weak inverse association between evening cortisol and wellbeing

(Table 2 and Figure 1B). Adjustment for body mass index, smoking and social class attenuated

this association. Again, there was no evidence of heterogeneity across studies (I^2=19.1; p for

heterogeneity =0.3).

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In the pooled analysis a flatter diurnal slope was associated with poorer wellbeing though this

was not statistically significant before or after adjustment for all covariates (Table 2 and Figure

1C). In sensitivity analysis, correcting for regression dilution bias, the sex, age and follow-up

time adjusted associations between cortisol and wellbeing were 0.036, -1.347 and -0.163 for

morning, evening and slope cortisol respectively.

DISCUSSION

Based on meta-analysis of individual participant data from four large cohort studies,

we found some evidence that higher evening cortisol was prospectively associated with lower

positive mental wellbeing in middle and older age. The magnitude of this association was

small (-0.06 standard deviations in wellbeing) in crude analysis though up to -0.17 standard

deviations in wellbeing accounting for possible regression dilution bias. This association was

attenuated by the inclusion of body mass index, smoking and socioeconomic position. It

remains unclear whether obesity is secondary to HPA axis dysregulation so this may represent

over-adjustment for a variable on the explanatory pathway, though obesity is not a key

determinant of wellbeing. Morning cortisol and diurnal slope were not associated with

wellbeing in the current study.

Evening cortisol is arguably the least affected by salivary sampling protocol deviations,

which can bias associations between cortisol and wellbeing towards the null.[7] Furthermore,

single-day sampling tends to bias cortisol estimates towards state rather than trait values,[30]

which may also explain the lack of association with wellbeing up to eight years later. Only one

of the included studies captured cortisol profiles on more than one day and this study only on

two days. We examined inter-individual differences in cortisol in relation to wellbeing up to

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eight years after assessment of cortisol patterns. It is possible that an association between cortisol and wellbeing would only be evident over a shorter lag time. When measured on the same day, studies have found higher positive affect among those with lower cortisol output.[14,34,35] In addition, lower output in the first 45 minutes after waking[8] and total output across the day[10] has been associated with higher wellbeing over a period of 3-4 weeks. Where intensive study designs have been used to measure intra-individual change based on serial measurements of both cortisol and wellbeing repeated over multiple days, some studies find evidence of an inverse association between cortisol output and positive affect[11,13,36-38] though others do not.[9,15,16] Trait positive affect has been found to predict higher evening cortisol (in men only)[39] though we are not aware that prospective association between baseline cortisol and subsequent mental wellbeing has been assessed. Future studies are warranted in order to examine the longitudinal association between mental wellbeing and cortisol sampled over multiple days to more accurately capture trait cortisol.

  Though the evidence remains inconsistent at present, explanatory pathways include the over-activation of the HPA axis which interacts with the serotonin system and may ultimately result in serotonin depletion, increasing proneness to negative emotional states and reducing positive emotionality.[6,40,41] In addition, it has been proposed that the energy-mobilising properties of cortisol may underlie an association with positive mental wellbeing and that this pathway may be less relevant for understanding an association between cortisol and mental ill health.[7] The evidence base is currently too limited to determine whether diurnal cortisol is related to different components of positive mental wellbeing and to negative mental health in the same way though there is some indication that wellbeing and illbeing do not show the same correlations with cortisol slope.[10] The current

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study used the Warwick Edinburgh Mental Wellbeing Scale, designed to capture both hedonic

and eudemonic wellbeing over the last two weeks. Future research might explore a wider set

of hedonic and eudemonic components of mental wellbeing, as well as measures of mental ill

health, in a single analytical sample to establish whether they show the same or different

relationships with diurnal cortisol.

Other limitations should be acknowledged. There were differences in the protocol for

the collection of cortisol although we did not find evidence of between-study heterogeneity in

the estimates from the meta-analysis (with all I^2 values below 21%). We used clock time but

cortisol patterns are more closely anchored to waking time. Participants were instructed to

provide samples at specified times post-waking but actual waking time was not recorded. In

addition, a maximum of four samples per day were collected and additional measures may

have provided more accurate assessment of diurnal cortisol, especially diurnal slope.

Nevertheless, the current study also has strengths. It includes a large number of

participants (ranging from 1756 to 7515 in each analysis and considerably larger than any

previous study), in population-based samples, using harmonized measures of wellbeing and

covariates. Individual participant data meta-analysis was used, which has advantages over

aggregate meta-analysis including greater statistical power and standardization of the

derivation of variables and analytical models.[42]

In summary, the findings from this meta-analysis do not provide support for the

hypothesis that cortisol profiles indicative of disrupted HPA axis functioning has strong

associations positive mental wellbeing in healthy middle-aged and older people. Of the three

diurnal cortisol levels considered here, only evening cortisol showed a prospective association

with wellbeing and only in minimally-adjusted analysis. However, cortisol was sampled on

only one or two days and studies with samples across multiple days may find stronger

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3   associations if they better characterize cortisol patterns, though it is likely that any
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However, neither CLS nor the UK Data Service bear any responsibility for the analysis or interpretation of these data.

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Table 1: Descriptive statistics of participants by study

	CaPS	HCS	NSHD	NCDS
Sample with WEMWBS and cortisol; n	592	1055	1736	5337
Gender; n(%) male	530 (100)	463 (44)	809 (47)	2464 (50)
Age range at cortisol measurement (years)	70 - 82	60 - 73	62 – 64	44 - 46
Age range at WEMWBS assessment (years); [mean (sd)]	73 - 90 [80.1 (3.9)]	69 – 78 [73.2 (2.4)]	62 - 64 [63.6 (0.8)]	50 - 51 [50.7(0.1)]
BMI (kg/m^2); mean(sd)	28.2 (3.8)	26.9 (4.2)	27.9 (4.8)	27.1 (4.7)
Adult current smoking status; n (%) smoker	53 (11.0)	84 (8.1)	160 (10.2)	893 (18.5)
Adult social class; n(%) manual	303 (59.5)	400 (39)	499 (29.2)	1626 (34.1)
WEMWBS score; mean (sd)	53.3 (10.6)	51.7 (8.1)	51.8 (8.0)	49.5 (7.9)
Sampling times; mean				
Waking sample	7.37AM	N/A	7.11AM	N/A
Waking + 30mins (+45 mins for NCDS)	8.13AM		7.42AM	8.11AM
Waking + 3h 45 mins	N/A		N/A	11:11AM
2pm sample	2.11PM		N/A	N/A
Evening sample	10.05PM		9.27PM	N/A
Serum cortisol (nmol/L)	N/A	258.1 (81.2)	N/A	N/A
Salivary cortisol (nmol/L)				
Early morning; mean (sd)	19.7 (9.5)		22.9 (9.6)	21.3 (10.8)
Night time; median (IQR)	2.3 (1.5, 3.5)		2.4 (1.7,3.7)	N/A
Diurnal slope (nmol/L/h); mean (sd)	-1.13 (0.7)		-1.43 (0.7)	-4.3 (3.8)
Cortisol Awakening Response; mean (sd)	2.3 (9.4)		6.4 (11.7)	N/A
Exclusions due to being on corticosteroids; n(%)	62 (10%)	13 (1.2%)	19(1.1%)	396 (7.5)[a]

[a]endocrine medication

Table 2. Overall summary estimates of effect for the associations between cortisol measures and wellbeing from a series of meta-analyses.

	Included cohorts	Number of individuals	Mean difference in WEMWBS score (95% CI) per SD increase in cortisol							
			Model 1[a]				Model 2[b]			
			Regression coefficient (95% CI)	P-value	Tests of hetero geneity I^2(%)	P-value[c]	Regression coefficient (95% CI)	P-value	Tests of hetero geneity I^2(%)	P-value[c]
Early morning cortisol	All	7515	0.02 (-0.17,0.21)	0.8	2.3	0.4	0.01 (-0.22,0.24)	0.9	18.0	0.3
Evening cortisol	CaPS NSHD	1756	-0.47 (-1.00,0.05)	0.08	20.3	0.3	-0.31 (-0.83,0.21)	0.2	19.1	0.3
Flatter diurnal slope	CaPS NSHD NCDS	6490	-0.07 (-0.27,0.14)	0.5	0.0	0.6	-0.08 (-0.28,0.13)	0.5	0.0	0.6

[a]sex, age at cortisol assessment, follow-up time to wellbeing assessment; [b]Model 1 plus body mass index, smoking status, adult social class; [c]p-values from Cochran's Q statistic performed as a test of between-study heterogeneity

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Figure 1. Meta-analysis of the association between wellbeing and (A) early morning cortisol; (B) evening cortisol; (C) flatter diurnal slope. All estimates are adjusted for sex and follow-up time.
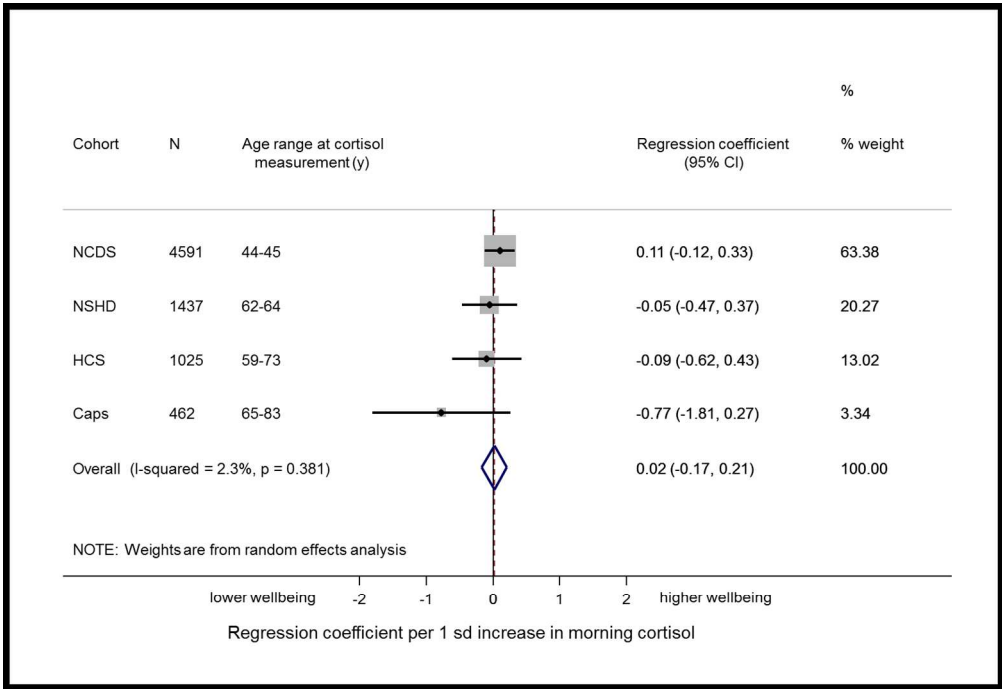
Figure 1A

Figure 1B

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Cohort	N	Age range at cortisol measurement (y)		Regression coefficient (95% CI)	% weight
NCDS	4318	44-45		-0.09 (-0.33, 0.14)	77.06
NSHD	1154	62-64		-0.06 (-0.53, 0.41)	19.53
Caps	416	65-83		0.51 (-0.61, 1.63)	3.41
Overall (I-squared = 0.0%, p = 0.583)				-0.07 (-0.27, 0.14)	100.00

NOTE: Weights are from random effects analysis

lower wellbeing   -2   -1   0   1   2   higher wellbeing

Regression coefficient per 1 sd increase in diurnal slope (flatter slope)

Figure 1C

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STROBE Statement—checklist of items that should be included in reports of observational studies

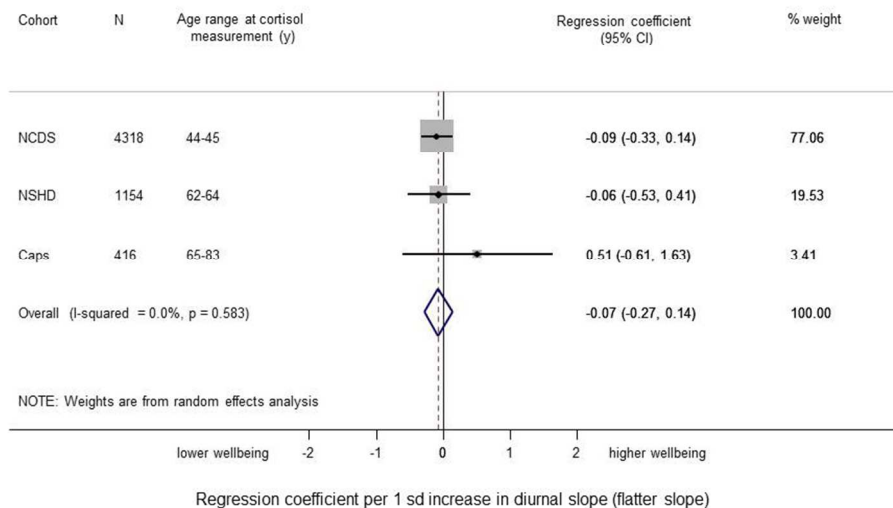|	Recommendation	Page
Title and abstract	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1,2
Introduction		
Background/rationale	Explain the scientific background and rationale for the investigation being reported	4
Objectives	State specific objectives, including any prespecified hypotheses	5
Methods		
Study design	Present key elements of study design early in the paper	5
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6
Participants	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5,6
	(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	Describe any efforts to address potential sources of bias	10
Study size	Explain how the study size was arrived at	9, Table 1
Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	(*a*) Describe all statistical methods, including those used to control for confounding	10
	(*b*) Describe any methods used to examine subgroups and interactions	10
	(*c*) Explain how missing data were addressed	10
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	
	(*e*) Describe any sensitivity analyses	10-11

Results		**Page**
Participants	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Table 1
	(b) Give reasons for non-participation at each stage	
	(c) Consider use of a flow diagram	N/A
Descriptive data	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
	(b) Indicate number of participants with missing data for each variable of interest	
	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	*Cohort study*—Report numbers of outcome events or summary measures over time	Table 1
Main results	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
	(*b*) Report category boundaries when continuous variables were categorized	
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion		
Key results	Summarise key results with reference to study objectives	13
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	Discuss the generalisability (external validity) of the study results	13
Other information		
Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24

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BMJ Open

Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

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Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

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Word count: 3313

Number of figures and tables: 3 figures, 2 tables

1

Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

ABSTRACT

Objectives: We conducted individual participant meta-analysis to test the hypothesis that cortisol patterns indicative of dysregulated HPA-axis functioning would be prospectively associated with poorer wellbeing at follow-up.

Setting: Four large UK-based cohort studies

Participants: Those providing valid salivary or serum cortisol samples (n=7515 for morning cortisol; n=1756 for evening cortisol) at baseline (age 44-82) and wellbeing data on the Warwick Edinburgh Mental Wellbeing Scale at follow-up (0-8 years) were included.

Results: Wellbeing was not associated with morning cortisol or diurnal slope though a borderline association with evening cortisol was found. Adjusting for sex and follow-up time, each 1 standard deviation increase in evening cortisol was associated with a -0.47 (95% CI -1.00, 0.05) point lower WEMWBS. This was attenuated by adjustment for body mass index, smoking and socioeconomic position. Between-study heterogeneity was low.

Conclusions: This study does not support the hypothesis that diurnal cortisol is prospectively associated with wellbeing up to 8 years later. However, replication in prospective studies with cortisol samples over multiple days is required.

Keywords: meta-analysis; individual participant data; positive psychology;

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Strengths

- Individual participant meta-analysis based on 1612-7515 participants with cortisol data

- Prospective study with up to 8 years of follow-up

- Validated mental wellbeing instrument based on 14 items capturing hedonic and eudaimonic components harmonized across cohorts

Limitations

- Salivary cortisol samples taken over 1 or 2 days; up to a maximum of 4 times per day

- Actual waking time was not recorded in all studies

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INTRODUCTION

Cortisol is a marker of hypothalamic-pituitary-adrenal (HPA) axis functioning and follows a diurnal rhythm. Large-scale epidemiological studies have measured salivary cortisol sampled several times during the course of a day to capture its rising levels during the awakening response and the subsequent decline across the day. The mental health and wellbeing consequences of raised cortisol levels are of interest particularly among older people given that some studies find they have higher evening cortisol levels[1] and greater total cortisol output throughout the day[2] compared with younger people though there is inter-individual variation in age-related change in cortisol [3] and this may reflect age-related change in disease rather than normal ageing [4].

Positive mental wellbeing is a multidimensional concept that captures hedonic (e.g. happiness and experiencing pleasure) and eudemonic (e.g. self-realization and psychologically functioning at potential) aspects of mental health that are distinct from depressive illness.[5] Cortisol may be linked to a positive psychological state through its effect on mood-altering neurotransmitters including serotonin.[6] Cortisol also has energy-mobilising properties that may in turn promote mental wellbeing.[7]

Two studies of healthy older people found no association between cortisol levels and positive wellbeing (captured by the positive items of the General Health Questionnaire[8] or by daily positive emotions[9]). A study of women aged 65 and over found that greater eudaimonic wellbeing was associated with flatter cortisol slope over the day, though this was due to lower morning cortisol levels which remained low across the day.[10] All studies were small (less than 200 participants). In younger adults (i.e. 55 years and younger), some studies

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have found that greater positive affect or happiness was associated with lower total cortisol

output[11-14] but others have not.[15,16] These studies have not examined prospective

associations between baseline cortisol patterns and wellbeing at follow-up in large,

population-based samples. Although stressors are typically associated with HPA axis

activation and decreases in vagal tone, some studies suggest that there may in some cases be

a subsequent response involving the dorso-vagal parasympathetic system and down-

regulation of the HPA axis resulting in low cortisol levels [17]. If this is the case then a long-

term inverse association between cortisol and wellbeing may not be evident.

The aim of the current study was to draw on individual participant data from four

large British cohort studies using meta-analysis to examine the longitudinal associations

between diurnal cortisol and positive mental wellbeing captured by an instrument which

summarizes positive thoughts and feelings from both hedonic and eudemonic perspectives.

We hypothesized that cortisol patterns indicative of disrupted HPA axis functioning (i.e. lower

early morning levels, higher evening levels and less steep decline (i.e. flatter response) across

the day) would be associated with lower wellbeing at follow-up.

METHODS

Cohort studies

We used the four cohort studies from the Healthy Ageing across the Life Course (HALCyon)

cross cohort research programme[18] with data on both cortisol and Warwick Edinburgh

Mental Wellbeing Scale: the Caerphilly Prospective Study (CaPS)[19]; the Hertfordshire Cohort

Study (HCS)[20]; the MRC National Survey of Health and Development (NSHD)[21]; and the

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National Child Development Study (NCDS).[22] All studies received appropriate ethical

approval.[19-22]

Caerphilly Prospective Study (CaPS)

CaPS is a cohort of men who were recruited when they were aged 45–59 years, between 1979

and 1983, from the town of Caerphilly and adjacent villages in South Wales. In the second

wave (1984 – 1988) the original cohort was supplemented with men of a similar age who had

moved into the defined area. Salivary cortisol was assessed at phase 5 of data collection in

2000–2004 when participants were aged 65 to 82 years and wellbeing was assessed in 2011

when they were aged 73 to 90 years.

Hertfordshire Cohort Study (HCS)

HCS is a cohort of men and women born in East, North or West Hertfordshire between 1931

and 1939 whose birth and infant records were available and who were alive and still living in

Hertfordshire in the 1990s. Cortisol was assessed at wave 1 of the data collection (1999–

2004) when participants were aged 60 – 73 years and wellbeing was assessed in 2008 when

they were aged 69 – 78.

The MRC National Survey of Health and Development (NSHD)

NSHD is a nationally representative sample of people born in England, Scotland, and Wales

during one week in March 1946 followed prospectively since birth. Cortisol and wellbeing

data were collected in 2008-10 when cohort members were aged 62-64 years.

The National Child Development Study (NCDS)

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NCDS is a nationally representative sample of people born in England, Scotland, and Wales

during 1 week in March 1958 followed prospectively since birth. Cortisol was assessed as part

of a bio-medical survey (2002–2004) when cohort members were aged 44-45 and wellbeing

was assessed at a follow-up (2008-2009) at mean age of 50.7.

Cortisol

All study members who had not withdrawn or been lost to follow-up at the relevant sweep

were invited to participate in the cortisol sampling. In HCS, one fasting morning serum cortisol

sample was taken from each participant at a research clinic between 8.30 and 9.30 AM,

frozen and subsequently measured by radioimmunoassay. Salivary cortisol samples were

collected in CaPS, NSHD and NCDS at multiple times across the day with participants shown

how to collect saliva using plain cotton wool swabs (salivettes) at home. Subjects were asked

to chew on the salivettes for 1-2 min and a saliva sample was obtained. In CaPS, participants

were requested to take samples on waking, 30 minutes later, at 2 pm and 10 pm on two

consecutive days. In NSHD, samples were taken on waking, 30 minutes later and at 9 pm.

Samples in NCDS were taken in the first 45 minutes after waking and 3 hours later. Samples

from CaPS, NSHD and NCDS were frozen and subsequently assayed by radioimmunoassay

done at the University of Dresden which specializes in high through-put cortisol assays.[23]

Mental wellbeing

Positive mental wellbeing was assessed using the Warwick Edinburgh Mental Wellbeing Scale

(WEMWBS) in all four studies. This self-completion scale captures positive affect, satisfying

interpersonal relationships and positive functioning. Items are worded positively and

respondents are asked to indicate how frequently, on a five point scale, they have

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experienced each statement over the last two weeks. Statements include "I've been feeling

good about myself", "I've been feeling close to other people", "I've been interested in new

things" and "I've been feeling optimistic about the future". Scores theoretically range from 14

to 70 with 70 indicating highest wellbeing. Where three or fewer items were missing, values

were imputed based on the average score for completed items (CaPS n=43; HCS n=52; NSHD

n=84; NCDS n=103). Internal consistency of the scale in all four cohorts was high (Cronbach

alpha=0.91 in HCS, NSHD and NCDS and 0.93 in CaPS.[24] Validation work indicates good

construct validity for a single factor structure as well as good criterion validity and test-retest

reliability and supports its use in general population samples.[25]

Covariates

Key covariates that might confound the association between cortisol and wellbeing and which

had been assessed in each of the cohorts were chosen *a priori*: sex, age at cortisol

measurement, follow-up time to measurement of wellbeing, body mass index (BMI), smoking,

and adult socio-economic position.[26-28] The covariates were measured at the same wave

as cortisol samples unless otherwise stated. BMI was calculated as measured weight divided

by the square of height and was categorized into quartiles when there was evidence of

deviation from linearity in the association with wellbeing (NSHD, NCDS). Smoking was

reported by participants and dichotomized into current smoker vs ex- and never smoker

(NCDS smoking data at age 42 years). Adult socioeconomic position was derived from own

occupational class (CaPS at age 47-67, HCS at age 60-73, NSHD at age 53 or earlier if missing,

NCDS at age 42) and grouped as manual or non-manual occupation (the latter indicating

greater socioeconomic advantage).

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Initial treatment of the data and standardization

Cortisol has a marked circadian rhythm and therefore the time of day at which it is sampled

will affect its level. In CaPS, NSHD and NCDS actual times when the salivary samples were

taken were recorded by participants. Observed values were adjusted for the time of sampling

by fitting a linear or polynomial function to the association between cortisol and time of

measurement and adding the resulting residuals from the best fit model to the overall mean

cortisol value. This gives the estimated cortisol level at the time specified in the protocol for

each participant. Morning cortisol (salivary or serum) levels were available in all four cohorts

(CaPS, HCS, NSHD and NCDS) and night time values in CaPS and NSHD. For NCDS, the later

cortisol measure was taken 3 hours after the morning measure on the same day (around

11.15AM) and there was no evening measure. However past publications support the notion

that the diurnal decline over this shorter period is a good surrogate for the decline from

morning till night[29] and hence this measure was used to derive a measure of diurnal slope.

HCS collected serum cortisol, the levels of which are around 20 times higher than the free

cortisol concentrations found in saliva. However, a study on the relationship between serum

and salivary cortisol in healthy individuals[30] showed that correlations were high whether

taken at the same time (>0.90) or 70 minutes apart (0.54—0.94). Those participants who

reported taking cortico-steroid medication were excluded from the analysis sample (CaPS

n=62; HCS n=13; NSHD n=19). In NCDS, participants were excluded from analysis if they

reported taking endocrine system medication (n= 396). Outlying salivary cortisol values

greater than 100 nmol/L were removed (CaPS n=5; NSHD n=7; NCDS n=21), since high cortisol

values can have substantial statistical influence on estimates and it is unclear what these high

values represent.[31] Morning salivary cortisol values that were not between 5am and noon

were removed and evening values if they were before 8pm, since these participants with

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atypical sleeping hours may have substantially different cortisol profiles. In CaPS, cortisol

values were averaged over the same measures obtained on two consecutive days. Early

morning salivary cortisol was computed in CaPS and NSHD as the mean of waking and 30 min

samples; to be comparable, in NCDS the cortisol measure taken within 45 minutes after

waking was used. In HCS morning serum cortisol was used.

To be able to combine the cohorts in a meta-analysis, the cortisol values were standardized

by deriving study-specific z-scores. In NCDS, both early and late morning cortisol were

positively skewed, as was night time cortisol in CaPS and NSHD, so values were $\log_e$

transformed before they were converted to z-scores. In addition to the early morning and

night time cortisol measures, we derived the diurnal slope (CaPS, NSHD, NCDS) as early

morning value subtracted from the evening (or late morning in NCDS) value and divided by

the lapsed time period. The overall slope is negative and so positive z-scores indicate a flatter

response.

Statistical Methods

A two stage meta-analysis was performed. In the first stage, we modelled WEMWBS as a

function of each diurnal cortisol indicator in turn using linear regression in each cohort

separately, with adjustment for i) sex, age at cortisol measurement and follow-up time to the

wellbeing measurement, ii) additional adjustment for BMI, smoking status and

socioeconomic position. There was no evidence of deviation from linearity in the association

between any of the cortisol measures and WEMWBS. There was no evidence of interaction

between sex and any of the cortisol measures. In sensitivity analysis to explore possible bias

arising from missing covariates , we adjusted for sex and age at cortisol and follow-up time i)

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using the maximum available sample with cortisol and wellbeing data (results not presented),

ii) using the sample restricted to only those participants that had complete data on all

covariates. Results did not materially differ for these two samples. In the second stage,

cohort-specific estimates were pooled in random-effects meta-analyses[32] chosen a priori

due to the expected heterogeneity between the different studies.

Sensitivity analyses

We corrected regression estimates for regression dilution bias arising from error in the

measurement of cortisol. The reliability ratios were estimated by regressing the cortisol

measure on day 2 on the measure on day 1 from CaPS data.[33] This yielded reliability ratios

of 0.554, 0.349 and 0.430 for morning, evening and slope cortisol respectively.

RESULTS

The characteristics of the participants of the four cohorts in the analysis are shown in Table 1.

Age at time of wellbeing measurement ranged from mean (sd) age of 50.7 (0.1) years in NCDS

to 80.1 (3.9) years in CaPS. Mean wellbeing ranged from 49.5 (7.9) in NCDS to 53.3 (10.6) in

CaPS and increased with mean age of the cohort. Mean early morning cortisol values were

similar for the three cohorts (CaPS, NSHD, NCDS) that had measured salivary cortisol. Night

time cortisol values were also similar although diurnal slope was more negative (indicating

greater decline) in NCDS than in NSHD and CaPS.

We found no evidence of an association between early morning cortisol and wellbeing in the

individual cohorts in sex, age and follow-up time adjusted models. The overall pooled estimate

was 0.02 (95% CI -0.17,0.21; p=0.8) and there was no evidence of heterogeneity in this

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association across studies (I^2=2.3; p for heterogeneity=0.4) (Table 2 and Figure 1A). Further

adjustment for all covariates did not affect the overall pooled estimate 0.01 (95% CI -0.22,0.24;

I^2=18.0; p for heterogeneity=0.3).

Sex, age and follow-up time adjusted associations between evening cortisol and wellbeing in

the individual cohorts were in the expected direction (i.e. higher evening cortisol was

associated with lower wellbeing) in NSHD: -0.33 (95% CI -0.77,0.11); CaPS -0.98 (95% CI -

2.03,0.07). This indicates a weak inverse association between evening cortisol and wellbeing

(Table 2 and Figure 1B). Adjustment for BMI, smoking and social class attenuated this

association. Again, there was no evidence of heterogeneity across studies (I^2=19.1; p for

heterogeneity =0.3).

In the pooled analysis a flatter diurnal slope was associated with poorer wellbeing though this

was not statistically significant before or after adjustment for all covariates (Table 2 and Figure

1C). Results excluding NCDS (based on decline in cortisol between early and late morning) were

similar (0.02 (95% CI -0.41, 0.46)).

In sensitivity analysis, correcting for regression dilution bias, the sex, age and follow-up time

adjusted associations between cortisol and wellbeing were 0.036, -1.347 and -0.163 for

morning, evening and slope cortisol respectively.

DISCUSSION

Based on meta-analysis of individual participant data from four large cohort studies,

we found that morning cortisol and diurnal slope were not associated with wellbeing but

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there was evidence that higher evening cortisol was prospectively associated with lower

positive mental wellbeing in middle and older age. The magnitude of this association was

small (-0.06 standard deviations in wellbeing) in crude analysis though up to -0.17 standard

deviations in wellbeing accounting for possible regression dilution bias. This association was

attenuated by the inclusion of BMI, smoking and socioeconomic position. It remains unclear

whether obesity is secondary to HPA axis dysregulation so this may represent over-

adjustment for a variable on the explanatory pathway. Perceived stress is also linked to lower

socioeconomic position, smoking and cortisol levels [27, 30] and so isolating an association

between cortisol and wellbeing independently of these factors needs to be interpreted with

caution.

Evening cortisol is arguably the least affected by salivary sampling protocol deviations,

which can bias associations between cortisol and wellbeing towards the null.[7] Furthermore,

single-day sampling tends to bias cortisol estimates towards state rather than trait values,[31]

which may also explain the lack of association with wellbeing up to eight years later. Only one

of the included studies captured cortisol profiles on more than one day and this study only on

two days. We examined inter-individual differences in cortisol in relation to wellbeing up to

eight years after assessment of cortisol patterns. It is possible that an association between

cortisol and wellbeing would only be evident over a shorter lag time. When measured on the

same day, studies have found higher positive affect among those with lower cortisol

output.[14,34,35] In addition, lower output in the first 45 minutes after waking[8] and total

output across the day[10] has been associated with higher wellbeing over a period of 3-4

weeks. Where intensive study designs have been used to measure intra-individual change

based on serial measurements of both cortisol and wellbeing repeated over multiple days,

some studies find evidence of an inverse association between cortisol output and positive

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affect[11,13,36-38] though others do not.[9,15,16] Trait positive affect has been found to

predict higher evening cortisol (in men only)[39] though we are not aware that a prospective

association between baseline cortisol and subsequent mental wellbeing has been assessed.

Future studies are warranted in order to examine the longitudinal association between

mental wellbeing and cortisol sampled over multiple days to more accurately capture trait

cortisol. In addition, cortisol samples taken at regular intervals throughout follow-up would

enable us to identify how changes in HPA-axis activation, such as hypocortisolism as a

response to chronic stress, might be related to mental wellbeing.

Though the evidence remains inconsistent at present, explanatory pathways include

the over-activation of the HPA axis which interacts with the serotonin system and may

ultimately result in serotonin depletion, increasing proneness to negative emotional states

and reducing positive emotionality.[6,40,41] In addition, it has been proposed that the

energy-mobilising properties of cortisol may underlie an association with positive mental

wellbeing and that this pathway may be less relevant for understanding an association

between cortisol and mental ill health.[7] The evidence base is currently too limited to

determine whether diurnal cortisol is related to different components of positive mental

wellbeing and to negative mental health in the same way though there is some indication that

wellbeing and illbeing do not show the same correlations with cortisol slope.[10] The current

study used the Warwick Edinburgh Mental Wellbeing Scale, designed to capture both hedonic

and eudemonic wellbeing over the last two weeks. Future research might explore a wider set

of hedonic and eudemonic components of mental wellbeing, as well as measures of mental ill

health, in a single analytical sample to establish whether they show the same or different

relationships with diurnal cortisol.

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Other limitations should be acknowledged. There were differences in the protocol for

the collection of cortisol although we did not find evidence of between-study heterogeneity in

the estimates from the meta-analysis (with all I^2 values below 21%). We used clock time but

cortisol patterns are more closely anchored to waking time. Participants were instructed to

provide samples at specified times post-waking but actual waking time was not recorded. In

addition, a maximum of four samples per day were collected and additional measures may

have provided more accurate assessment of diurnal cortisol, especially diurnal slope. We did

not consider factors that may modify the prospective association between cortisol and

wellbeing, such as social support which has been shown to buffer the health impact of

stress.[42]

Nevertheless, the current study also has strengths. It includes a large number of

participants (ranging from 1756 to 7515 in each analysis and considerably larger than any

previous study), in population-based samples, using harmonized measures of wellbeing and

covariates. Individual participant data meta-analysis was used, which has advantages over

aggregate meta-analysis including greater statistical power and standardization of the

derivation of variables and analytical models.[43]

In summary, the findings from this meta-analysis do not provide support for the

hypothesis that cortisol profiles indicative of disrupted HPA axis functioning has strong

associations positive mental wellbeing in healthy middle-aged and older people. Of the three

diurnal cortisol levels considered here, only evening cortisol showed a prospective association

with wellbeing and only in minimally-adjusted analysis. However, cortisol was sampled on

only one or two days and studies with samples across multiple days may find stronger

associations if they better characterize cortisol patterns, though it is likely that any

associations, if found, will be of modest to moderate magnitude.

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Table 1: Descriptive statistics of participants by study

	CaPS	HCS	NSHD	NCDS
Sample with WEMWBS and cortisol; n	592	1055	1736	5337
Gender; n(%) male	530 (100)	463 (44)	809 (47)	2464 (50)
Age range at cortisol measurement (years); [mean (sd)]	65 – 82 [72.8 (4.0)]	60 – 73 [66.6 (2.7)]	62 – 64 [63.6 (0.8)]	44 – 46 [44.1 (0.2)]
Age range at WEMWBS assessment (years); [mean (sd)]	73 - 90 [80.1 (3.9)]	69 – 78 [73.2 (2.4)]	62 - 64 [63.6 (0.8)]	50 – 51 [50.7(0.1)]
Follow-up time cortisol to WEMWBS assessment (years); mean (sd)	7.6 (0.4)	6.6 (1.2)	0	6.6 (0.3)
BMI (kg/m^2); mean(sd)	28.2 (3.8)	26.9 (4.2)	27.9 (4.8)	27.1 (4.7)
Adult current smoking status; n (%) smoker	53 (11.0)	84 (8.1)	160 (10.2)	893 (18.5)
Adult social class; n(%) manual	303 (59.5)	400 (39)	499 (29.2)	1626 (34.1)
WEMWBS score; mean (sd)	53.3 (10.6)	51.7 (8.1)	51.8 (8.0)	49.5 (7.9)
Sampling times; mean				
Waking sample	7.37AM	N/A	7.11AM	N/A
Waking + 30mins (+45 mins for NCDS)	8.13AM		7.42AM	8.11AM
Waking + 3h 45 mins	N/A		N/A	11:11AM
2pm sample	2.11PM		N/A	N/A
Evening sample	10.05PM		9.27PM	N/A
Serum cortisol (nmol/L)	N/A	258.1 (81.2)	N/A	N/A
Salivary cortisol (nmol/L)				
Early morning; mean (sd)	19.7 (9.5)		22.9 (9.6)	21.3 (10.8)
Night time; median (IQR)	2.3 (1.5, 3.5)		2.4 (1.7,3.7)	N/A
Diurnal slope (nmol/L/h); mean (sd)	-1.13 (0.7)		-1.43 (0.7)	-4.3 (3.8)
Exclusions due to being on corticosteroids; n(%)	62 (10%)	13 (1.2%)	19(1.1%)	396 (7.5)[a]

[a]endocrine medication

Table 2. Overall summary estimates of effect for the associations between cortisol measures and wellbeing from a series of meta-analyses.

	Included cohorts	Number of individuals	Mean difference in WEMWBS score (95% CI) per SD increase in cortisol							
			Model 1[a]				Model 2[b]			
			Regression coefficient (95% CI)	P-value	Tests of hetero geneity I^2(%)	P-value[c]	Regression coefficient (95% CI)	P-value	Tests of hetero geneity I^2(%)	P-value[c]
Early morning cortisol	All	7515	0.02 (-0.17,0.21)	0.8	2.3	0.4	0.01 (-0.22,0.24)	0.9	18.0	0.3
Evening cortisol	CaPS NSHD	1756	-0.47 (-1.00,0.05)	0.08	20.3	0.3	-0.31 (-0.83,0.21)	0.2	19.1	0.3
Flatter diurnal slope	CaPS NSHD NCDS	6490	-0.07 (-0.27,0.14)	0.5	0.0	0.6	-0.08 (-0.28,0.13)	0.5	0.0	0.6

[a]sex, age at cortisol assessment, follow-up time to wellbeing assessment; [b]Model 1 plus body mass index, smoking status, adult social class; [c]p-values from Cochran's Q statistic performed as a test of between-study heterogeneity

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Figure Legend

Figure 1. Meta-analysis of the association between wellbeing and (A) early morning cortisol;

(B) evening cortisol; (C) flatter diurnal slope. All estimates are adjusted for sex and follow-up
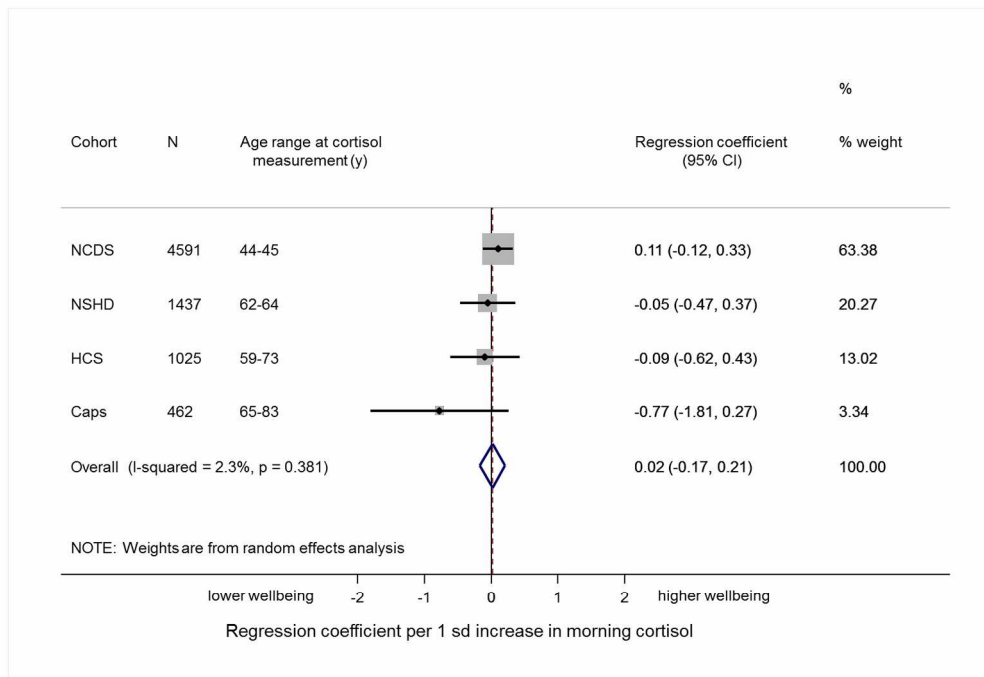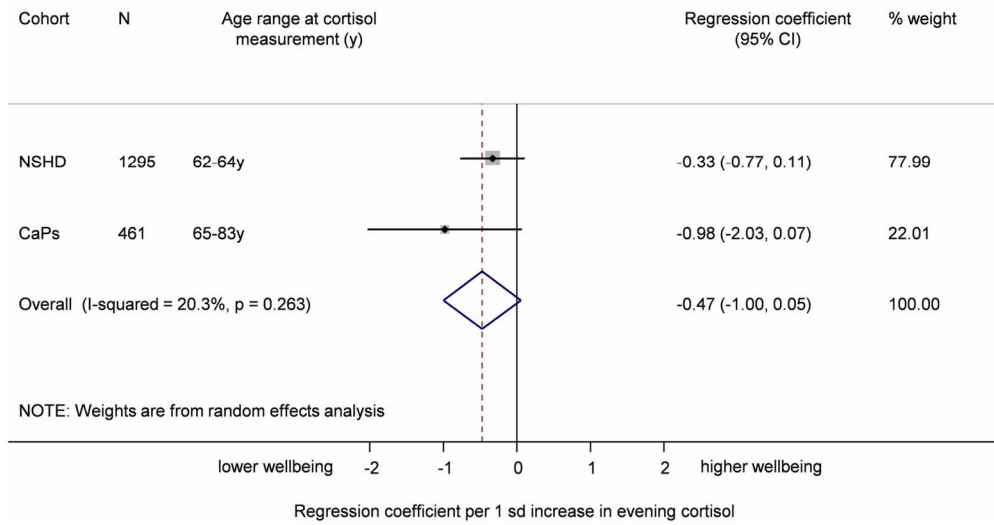
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Figure 1A

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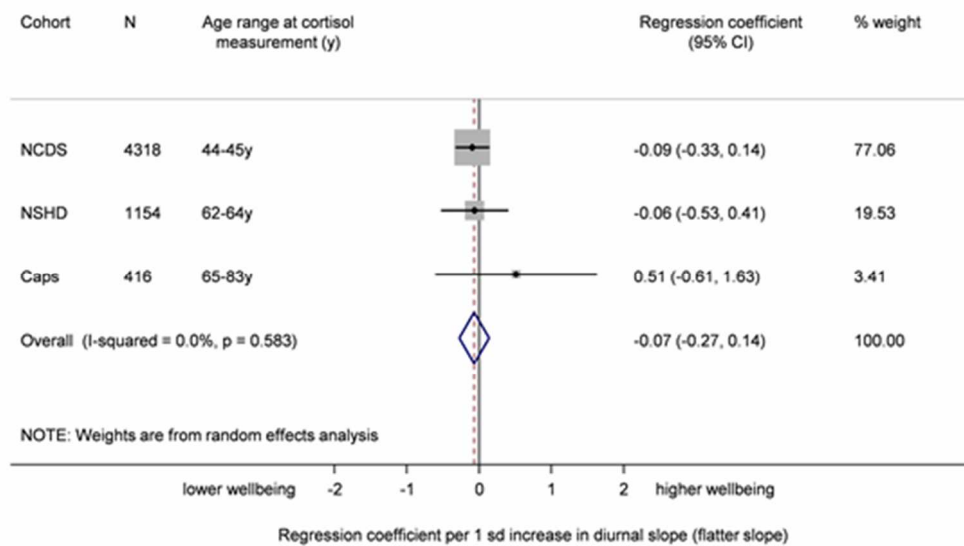41
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Cohort	N	Age range at cortisol measurement (y)		Regression coefficient (95% CI)	% weight
NSHD	1295	62-64y		-0.33 (-0.77, 0.11)	77.99
CaPs	461	65-83y		-0.98 (-2.03, 0.07)	22.01
Overall (I-squared = 20.3%, p = 0.263)				-0.47 (-1.00, 0.05)	100.00

NOTE: Weights are from random effects analysis

lower wellbeing   -2   -1   0   1   2   higher wellbeing

Regression coefficient per 1 sd increase in evening cortisol

137x74mm (300 x 300 DPI)

Cohort	N	Age range at cortisol measurement (y)		Regression coefficient (95% CI)	% weight
NCDS	4318	44-45y		-0.09 (-0.33, 0.14)	77.06
NSHD	1154	62-64y		-0.06 (-0.53, 0.41)	19.53
Caps	416	65-83y		0.51 (-0.61, 1.63)	3.41
Overall (I-squared = 0.0%, p = 0.583)				-0.07 (-0.27, 0.14)	100.00

NOTE: Weights are from random effects analysis

lower wellbeing   -2   -1   0   1   2   higher wellbeing

Regression coefficient per 1 sd increase in diurnal slope (flatter slope)

Figure 1C

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Recommendation	Page
Title and abstract	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1,2
Introduction		
Background/rationale	Explain the scientific background and rationale for the investigation being reported	4
Objectives	State specific objectives, including any prespecified hypotheses	5
Methods		
Study design	Present key elements of study design early in the paper	5
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6
Participants	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5,6
	(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	Describe any efforts to address potential sources of bias	9-10
Study size	Explain how the study size was arrived at	9, Table 1
Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	(*a*) Describe all statistical methods, including those used to control for confounding	10
	(*b*) Describe any methods used to examine subgroups and interactions	10
	(*c*) Explain how missing data were addressed	10
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	
	(*e*) Describe any sensitivity analyses	11

Continued on next page

Results		**Page**
Participants	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Table 1
	(b) Give reasons for non-participation at each stage	
	(c) Consider use of a flow diagram	N/A
Descriptive data	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
	(b) Indicate number of participants with missing data for each variable of interest	
	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	*Cohort study*—Report numbers of outcome events or summary measures over time	Table 1
Main results	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
	(*b*) Report category boundaries when continuous variables were categorized	
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion		
Key results	Summarise key results with reference to study objectives	13
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13-14
Generalisability	Discuss the generalisability (external validity) of the study results	14
Other information		
Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

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BMJ Open

Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

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Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

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Number of figures and tables: 4 figures, 2 tables

1

Diurnal cortisol and mental wellbeing in middle and older age: evidence from four cohort studies

ABSTRACT

Objectives: We conducted individual participant meta-analysis to test the hypothesis that cortisol patterns indicative of dysregulated HPA-axis functioning would be prospectively associated with poorer wellbeing at follow-up.

Setting: Four large UK-based cohort studies

Participants: Those providing valid salivary or serum cortisol samples (n=7515 for morning cortisol; n=1612 for cortisol awakening response) at baseline (age 44-82) and wellbeing data on the Warwick Edinburgh Mental Wellbeing Scale at follow-up (0-8 years) were included.

Results: Wellbeing was not associated with morning cortisol, diurnal slope or awakening response though a borderline association with evening cortisol was found. Adjusting for sex and follow-up time, each 1 standard deviation increase in evening cortisol was associated with a -0.47 (95% CI -1.00, 0.05) point lower wellbeing. This was attenuated by adjustment for body mass index, smoking and socioeconomic position. Between-study heterogeneity was low.

Conclusions: This study does not support the hypothesis that diurnal cortisol is prospectively associated with wellbeing up to 8 years later. However, replication in prospective studies with cortisol samples over multiple days is required.

Keywords: meta-analysis; individual participant data; positive psychology;

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Strengths

- Individual participant meta-analysis based on 1612-7515 participants with cortisol

  data

- Prospective study with up to 8 years of follow-up

- Validated mental wellbeing instrument based on 14 items capturing hedonic and

  eudaimonic components harmonized across cohorts

Limitations

- Salivary cortisol samples taken over 1 or 2 days; up to a maximum of 4 times per day

- Actual waking time was not recorded in all studies

3

INTRODUCTION

Cortisol is a marker of hypothalamic-pituitary-adrenal (HPA) axis functioning and

follows a diurnal rhythm. Large-scale epidemiological studies have measured salivary cortisol

sampled several times during the course of a day to capture its rising levels during the

awakening response and the subsequent decline across the day. The mental health and

wellbeing consequences of raised cortisol levels are of interest particularly among older

people given that some studies find they have higher evening cortisol levels[1] and greater

total cortisol output throughout the day[2] compared with younger people though there is

inter-individual variation in age-related change in cortisol [3] and this may reflect age-related

change in disease rather than normal ageing [4].

Positive mental wellbeing is a multidimensional concept that captures hedonic (e.g.

happiness and experiencing pleasure) and eudemonic (e.g. self-realization and psychologically

functioning at potential) aspects of mental health that are distinct from depressive illness.[5]

Cortisol may be linked to a positive psychological state through its effect on mood-altering

neurotransmitters including serotonin.[6] Cortisol also has energy-mobilising properties that

may in turn promote mental wellbeing.[7]

Two studies of healthy older people found no association between cortisol levels and

positive wellbeing (captured by the positive items of the General Health Questionnaire[8] or

by daily positive emotions[9]). A study of women aged 65 and over found that greater

eudaimonic wellbeing was associated with flatter cortisol slope over the day, though this was

due to lower morning cortisol levels which remained low across the day.[10] All studies were

small (less than 200 participants). In younger adults (i.e. 55 years and younger), some studies

have found that greater positive affect or happiness was associated with lower total cortisol

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output[11-14] but others have not.[15,16] Greater optimism has also been associated with a smaller awakening response.[12]

These studies have not examined prospective associations between baseline cortisol patterns and wellbeing at follow-up in large, population-based samples. Although stressors are typically associated with HPA axis activation and decreases in vagal tone, some studies suggest that there may in some cases be a subsequent response involving the dorso-vagal parasympathetic system and down-regulation of the HPA axis resulting in low cortisol levels [17]. If this is the case then a long-term inverse association between cortisol and wellbeing may not be evident.

The aim of the current study was to draw on individual participant data from four large British cohort studies using meta-analysis to examine the longitudinal associations between diurnal cortisol and positive mental wellbeing captured by an instrument which summarizes positive thoughts and feelings from both hedonic and eudemonic perspectives. We hypothesized that cortisol patterns indicative of disrupted HPA axis functioning (i.e. lower early morning levels, higher evening levels, less steep decline (i.e. flatter response) across the day and larger awakening response) would be associated with lower wellbeing at follow-up.

METHODS

Cohort studies

We used the four cohort studies from the Healthy Ageing across the Life Course (HALCyon) cross cohort research programme[18] with data on both cortisol and Warwick Edinburgh Mental Wellbeing Scale (WEMWBS): the Caerphilly Prospective Study (CaPS)[19]; the Hertfordshire Cohort Study (HCS)[20]; the MRC National Survey of Health and Development

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(NSHD)[21]; and the National Child Development Study (NCDS).[22] All studies received

appropriate ethical approval.[19-22]

Caerphilly Prospective Study (CaPS)

CaPS is a cohort of men who were recruited when they were aged 45–59 years, between 1979

and 1983, from the town of Caerphilly and adjacent villages in South Wales. In the second

wave (1984 – 1988) the original cohort was supplemented with men of a similar age who had

moved into the defined area. Salivary cortisol was assessed at phase 5 of data collection in

2000–2004 when participants were aged 65 to 82 years and wellbeing was assessed in 2011

when they were aged 73 to 90 years.

Hertfordshire Cohort Study (HCS)

HCS is a cohort of men and women born in East, North or West Hertfordshire between 1931

and 1939 whose birth and infant records were available and who were alive and still living in

Hertfordshire in the 1990s. Cortisol was assessed at wave 1 of the data collection (1999–

2004) when participants were aged 60 – 73 years and wellbeing was assessed in 2008 when

they were aged 69 – 78.

The MRC National Survey of Health and Development (NSHD)

NSHD is a nationally representative sample of people born in England, Scotland, and Wales

during one week in March 1946 followed prospectively since birth. Cortisol and wellbeing

data were collected in 2008-10 when cohort members were aged 62-64 years.

The National Child Development Study (NCDS)

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NCDS is a nationally representative sample of people born in England, Scotland, and Wales

during 1 week in March 1958 followed prospectively since birth. Cortisol was assessed as part

of a bio-medical survey (2002–2004) when cohort members were aged 44-45 and wellbeing

was assessed at a follow-up (2008-2009) at mean age of 50.7.

Cortisol

All study members who had not withdrawn or been lost to follow-up at the relevant sweep

were invited to participate in the cortisol sampling. In HCS, one fasting morning serum cortisol

sample was taken from each participant at a research clinic between 8.30 and 9.30 AM (the

exact timing was not recorded), frozen and subsequently measured by radioimmunoassay.

Salivary cortisol samples were collected in CaPS, NSHD and NCDS at multiple times across the

day with participants shown how to collect saliva using plain cotton wool swabs (salivettes) at

home. Subjects were asked to chew on the salivettes for 1-2 min and a saliva sample was

obtained. In CaPS, participants were requested to take samples on waking, 30 minutes later,

at 2 pm and 10 pm on two consecutive days. In NSHD, samples were taken on waking, 30

minutes later and at 9 pm. Samples in NCDS were taken in the first 45 minutes after waking

and 3 hours later. Samples from CaPS, NSHD and NCDS were frozen and subsequently assayed

by radioimmunoassay done at the University of Dresden which specializes in high through-put

cortisol assays.[23]

Mental wellbeing

Positive mental wellbeing was assessed using WEMWBS in all four studies. This self-

completion scale captures positive affect, satisfying interpersonal relationships and positive

functioning. Items are worded positively and respondents are asked to indicate how

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frequently, on a five point scale, they have experienced each statement over the last two

weeks. Statements include "I've been feeling good about myself", "I've been feeling close to

other people", "I've been interested in new things" and "I've been feeling optimistic about

the future". Scores theoretically range from 14 to 70 with 70 indicating highest wellbeing.

Where three or fewer items were missing, values were imputed based on the average score

for completed items (CaPS n=43; HCS n=52; NSHD n=84; NCDS n=103). Internal consistency of

the scale in all four cohorts was high (Cronbach alpha=0.91 in HCS, NSHD and NCDS and 0.93

in CaPS.[24] Validation work indicates good construct validity for a single factor structure as

well as good criterion validity and test-retest reliability and supports its use in general

population samples.[25]

Covariates

Key covariates that might confound the association between cortisol and wellbeing and which

had been assessed in each of the cohorts were chosen *a priori*: sex, age at cortisol

measurement, follow-up time to measurement of wellbeing, body mass index (BMI), smoking,

and adult socio-economic position.[26-28] The covariates were measured at the same wave

as cortisol samples unless otherwise stated. BMI was calculated as measured weight divided

by the square of height and was categorized into quartiles when there was evidence of

deviation from linearity in the association with wellbeing (NSHD, NCDS). Smoking was

reported by participants and dichotomized into current smoker vs ex- and never smoker

(NCDS smoking data at age 42 years). Adult socioeconomic position was derived from own

occupational class (CaPS at age 47-67, HCS at age 60-73, NSHD at age 53 or earlier if missing,

NCDS at age 42) and grouped as manual or non-manual occupation (the latter indicating

greater socioeconomic advantage).

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Initial treatment of the data and standardization

Cortisol has a marked circadian rhythm and therefore the time of day at which it is sampled

will affect its level. In CaPS, NSHD and NCDS actual times when the salivary samples were

taken were recorded by participants. Observed values were adjusted for the time of sampling

by fitting a linear or polynomial function to the association between cortisol and time of

measurement and adding the resulting residuals from the best fit model to the overall mean

cortisol value. This gives the estimated cortisol level at the time specified in the protocol for

each participant. Morning cortisol (salivary or serum) levels were available in all four cohorts

(CaPS, HCS, NSHD and NCDS) and night time values in CaPS and NSHD. For NCDS, the later

cortisol measure was taken 3 hours after the morning measure on the same day (around

11.15AM) and there was no evening measure. However past publications support the notion

that the diurnal decline over this shorter period is a good surrogate for the decline from

morning till night[29] and hence this measure was used to derive a measure of diurnal slope.

HCS collected serum cortisol, the levels of which are around 20 times higher than the free

cortisol concentrations found in saliva. However, a study on the relationship between serum

and salivary cortisol in healthy individuals[30] showed that correlations were high whether

taken at the same time (>0.90) or 70 minutes apart (0.54—0.94). Those participants who

reported taking cortico-steroid medication were excluded from the analysis sample (CaPS

n=62; HCS n=13; NSHD n=19). In NCDS, participants were excluded from analysis if they

reported taking endocrine system medication (n= 396). Outlying salivary cortisol values

greater than 100 nmol/L were removed (CaPS n=5; NSHD n=7; NCDS n=21), since high cortisol

values can have substantial statistical influence on estimates and it is unclear what these high

values represent.[31] Morning salivary cortisol values that were not between 5am and noon

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were removed and evening values if they were before 8pm, since these participants with

atypical sleeping hours may have substantially different cortisol profiles. In CaPS, cortisol

values were averaged over the same measures obtained on two consecutive days. Early

morning salivary cortisol was computed in CaPS and NSHD as the mean of waking and 30 min

samples; to be comparable, in NCDS the cortisol measure taken within 45 minutes after

waking was used. In HCS morning serum cortisol was used.

To be able to combine the cohorts in a meta-analysis, the cortisol values were standardized

by deriving study-specific z-scores. In NCDS, both early and late morning cortisol were

positively skewed, as was night time cortisol in CaPS and NSHD, so values were $\log_e$

transformed before they were converted to z-scores. In addition to the early morning and

night time cortisol measures, we derived the diurnal slope (CaPS, NSHD, NCDS) as early

morning value subtracted from the evening (or late morning in NCDS) value and divided by

the lapsed time period. The overall slope is negative and so positive z-scores indicate a flatter

response. The cortisol awakening response (CAR) was derived as the difference between 30

minute post waking sample and the waking sample (CaPS, NSHD).

Statistical Methods

A two stage meta-analysis was performed. In the first stage, we modelled WEMWBS as a

function of each continuous diurnal cortisol indicator in turn using linear regression in each

cohort separately, with adjustment for i) sex, age at cortisol measurement and follow-up time

to the wellbeing measurement, ii) additional adjustment for BMI, smoking status and

socioeconomic position. There was no evidence of deviation from linearity in the association

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between any of the cortisol measures and WEMWBS. There was no evidence of interaction between sex and any of the cortisol measures. In sensitivity analysis to explore possible bias arising from missing covariates , we adjusted for sex and age at cortisol measurement and follow-up time i) using the maximum available sample with cortisol and wellbeing data (results not presented), ii) using the sample restricted to only those participants that had complete data on all covariates. Results did not materially differ for these two samples. In the second stage, cohort-specific estimates were pooled in random-effects meta-analyses[32] chosen a priori due to the expected heterogeneity between the different studies.

Sensitivity analyses

We corrected regression estimates for regression dilution bias arising from error in the measurement of cortisol. The reliability ratios were estimated by regressing the cortisol measure on day 2 on the measure on day 1 from CaPS data.[33] This yielded reliability ratios of 0.554, 0.349 and 0.430 for morning, evening and slope cortisol respectively. In addition, because both heightened and blunted CAR have been linked to raised disease risk, we tested whether wellbeing differed for those with a CAR in the top third or bottom third relative to the middle third.

RESULTS

The characteristics of the participants of the four cohorts in the analysis are shown in Table 1. Age at time of wellbeing measurement ranged from mean (sd) age of 50.7 (0.1) years in NCDS to 80.1 (3.9) years in CaPS. Mean wellbeing ranged from 49.5 (7.9) in NCDS to 53.3 (10.6) in CaPS and increased with mean age of the cohort. Mean early morning cortisol values were similar for the three cohorts (CaPS, NSHD, NCDS) that had measured salivary cortisol. Night

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time cortisol values were also similar although diurnal slope was more negative (indicating

greater decline) in NCDS than in NSHD and CaPS.

We found no evidence of an association between early morning cortisol and wellbeing in the

individual cohorts in sex, age and follow-up time adjusted models. The overall pooled estimate

was 0.02 (95% CI -0.17,0.21; p=0.8) and there was no evidence of heterogeneity in this

association across studies (I^2=2.3; p for heterogeneity=0.4) (Table 2 and Figure 1). Further

adjustment for all covariates did not affect the overall pooled estimate 0.01 (95% CI -0.22,0.24;

I^2=18.0; p for heterogeneity=0.3).

Sex, age and follow-up time adjusted associations between evening cortisol and wellbeing in

the individual cohorts were in the expected direction (i.e. higher evening cortisol was

associated with lower wellbeing) in NSHD: -0.33 (95% CI -0.77,0.11); CaPS -0.98 (95% CI -

2.03,0.07). This indicates a weak inverse association between evening cortisol and wellbeing

(Table 2 and Figure 2). Adjustment for BMI, smoking and social class attenuated this

association. Again, there was no evidence of heterogeneity across studies (I^2=19.1; p for

heterogeneity =0.3).

In the pooled analysis a flatter diurnal slope was associated with poorer wellbeing though this

was not statistically significant before or after adjustment for all covariates (Table 2 and Figure

3). Results excluding NCDS (based on decline in cortisol between early and late morning) were

similar (0.02 (95% CI -0.41, 0.46)). In the sex, age and follow-up time adjusted and in the fully

adjusted models, a higher CAR tended to be associated with lower wellbeing although the

association did not approach statistical significance (Table 2 and Figure 4).

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In sensitivity analysis, correcting for regression dilution bias, the sex, age and follow-up time adjusted associations between cortisol and wellbeing were 0.036, -1.347 and -0.163 for morning, evening and slope cortisol respectively. We found no evidence that wellbeing differed according to thirds of the CAR distribution.

DISCUSSION

Based on meta-analysis of individual participant data from four large cohort studies, we found that morning cortisol, diurnal slope and CAR were not associated with wellbeing but there was evidence that higher evening cortisol was prospectively associated with lower positive mental wellbeing in middle and older age. The magnitude of this association was small (-0.06 standard deviations in wellbeing) in crude analysis though up to -0.17 standard deviations in wellbeing accounting for possible regression dilution bias. This association was attenuated by the inclusion of BMI, smoking and socioeconomic position. It remains unclear whether obesity is secondary to HPA axis dysregulation so this may represent over-adjustment for a variable on the explanatory pathway. Perceived stress is also linked to lower socioeconomic position, smoking and cortisol levels [27, 30] and so isolating an association between cortisol and wellbeing independently of these factors needs to be interpreted with caution.

Evening cortisol is arguably the least affected by salivary sampling protocol deviations, which can bias associations between cortisol and wellbeing towards the null.[7] Furthermore, single-day sampling tends to bias cortisol estimates towards state rather than trait values,[31] which may also explain the lack of association with wellbeing up to eight years later. Only one of the included studies captured cortisol profiles on more than one day and this study (that is,

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CaPS) only sampled cortisol on two days. We note that there was also a trend towards lower

wellbeing among those with higher morning cortisol in CaPS though this did not attain

statistical significance. Replication in additional studies with samples over multiple days is

warranted. We examined inter-individual differences in cortisol in relation to wellbeing up to

eight years after assessment of cortisol patterns. It is possible that an association between

cortisol and wellbeing would only be evident over a shorter lag time. When measured on the

same day, studies have found higher positive affect among those with lower cortisol

output.[14,34,35] In addition, lower output in the first 45 minutes after waking[8] and total

output across the day[10] has been associated with higher wellbeing over a period of 3-4

weeks. Where intensive study designs have been used to measure intra-individual change

based on serial measurements of both cortisol and wellbeing repeated over multiple days,

some studies find evidence of an inverse association between cortisol output and positive

affect[11,13,36-38] though others do not.[9,15,16] Trait positive affect has been found to

predict higher evening cortisol (in men only)[39] though we are not aware that a prospective

association between baseline cortisol and subsequent mental wellbeing has been assessed.

Future studies are warranted in order to examine the longitudinal association between

mental wellbeing and cortisol sampled over multiple days to more accurately capture trait

cortisol. In addition, cortisol samples taken at regular intervals throughout follow-up would

enable us to identify how changes in HPA-axis activation, such as hypocortisolism as a

response to chronic stress, might be related to mental wellbeing.

Though the evidence remains inconsistent at present, explanatory pathways include

the over-activation of the HPA axis which interacts with the serotonin system and may

ultimately result in serotonin depletion, increasing proneness to negative emotional states

and reducing positive emotionality.[6,40,41] In addition, it has been proposed that the

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energy-mobilising properties of cortisol may underlie an association with positive mental

wellbeing and that this pathway may be less relevant for understanding an association

between cortisol and mental ill health.[7] The evidence base is currently too limited to

determine whether diurnal cortisol is related to different components of positive mental

wellbeing and to negative mental health in the same way though there is some indication that

wellbeing and illbeing do not show the same correlations with cortisol slope.[10] The current

study used WEMWBS, designed to capture both hedonic and eudemonic wellbeing over the

last two weeks. Future research might explore a wider set of hedonic and eudemonic

components of mental wellbeing, as well as measures of mental ill health, in a single

analytical sample to establish whether they show the same or different relationships with

diurnal cortisol.

Other limitations should be acknowledged. There were differences in the protocol for

the collection of cortisol. Morning serum cortisol was collected in one study and salivary

cortisol in the remaining three. Among those studies with salivary cortisol, morning average

cortisol was derived from samples taken at or before the typical peak (CaPS and NSHD) but

after the typical peak in NCDS, and cortisol levels differ considerably during this period.[42] In

addition, we excluded participants taking endocrine system medication in NCDS in contrast to

participants on cortico-steroid medication in the other studies though the former approach is

more conservative. Despite these differences we did not find evidence of between-study

heterogeneity in the estimates from the meta-analysis (with all I^2 values below 21%). We used

clock time but cortisol patterns are more closely anchored to waking time. Participants were

instructed to provide samples at specified times post-waking but actual waking time was not

recorded. In addition, a maximum of four samples per day were collected and additional

measures may have provided more accurate assessment of diurnal cortisol, especially diurnal

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slope. We did not consider factors that may modify the prospective association between cortisol and wellbeing, such as social support which has been shown to buffer the health impact of stress.[43]

Nevertheless, the current study also has strengths. It includes a large number of participants (ranging from 1756 to 7515 in each analysis and considerably larger than any previous study), in population-based samples, using harmonized measures of wellbeing and covariates. Individual participant data meta-analysis was used, which has advantages over aggregate meta-analysis including greater statistical power and standardization of the derivation of variables and analytical models.[44]

In summary, the findings from this meta-analysis do not provide support for the hypothesis that cortisol profiles indicative of disrupted HPA axis functioning has strong associations positive mental wellbeing in healthy middle-aged and older people. Of the four diurnal cortisol levels considered here, only evening cortisol showed a prospective association with wellbeing and only in minimally-adjusted analysis. However, cortisol was sampled on only one or two days and studies with samples across multiple days may find stronger associations if they better characterize cortisol patterns, though it seems likely that any associations, if found, will be of modest to moderate magnitude.

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Table 1: Descriptive statistics of participants by study

	CaPS	HCS	NSHD	NCDS
Sample with WEMWBS and cortisol; n	592	1055	1736	5337
Gender; n(%) male	530 (100)	463 (44)	809 (47)	2464 (50)
Age range at cortisol measurement (years); [mean (sd)]	65 – 82 [72.8 (4.0)]	60 – 73 [66.6 (2.7)]	62 – 64 [63.6 (0.8)]	44 – 46 [44.1 (0.2)]
Age range at WEMWBS assessment (years); [mean (sd)]	73 - 90 [80.1 (3.9)]	69 – 78 [73.2 (2.4)]	62 - 64 [63.6 (0.8)]	50 – 51 [50.7(0.1)]
Follow-up time cortisol to WEMWBS assessment (years); mean (sd)	7.6 (0.4)	6.6 (1.2)	0	6.6 (0.3)
BMI (kg/m^2); mean(sd)	28.2 (3.8)	26.9 (4.2)	27.9 (4.8)	27.1 (4.7)
Adult current smoking status; n (%) smoker	53 (11.0)	84 (8.1)	160 (10.2)	893 (18.5)
Adult social class; n(%) manual	303 (59.5)	400 (39)	499 (29.2)	1626 (34.1)
WEMWBS score; mean (sd)	53.3 (10.6)	51.7 (8.1)	51.8 (8.0)	49.5 (7.9)
Sampling times; mean				
Waking sample	7.37AM	N/A	7.11AM	N/A
Waking + 30mins (+45 mins for NCDS)	8.13AM		7.42AM	8.11AM
Waking + 3h 45 mins	N/A		N/A	11:11AM
2pm sample	2.11PM		N/A	N/A
Evening sample	10.05PM		9.27PM	N/A
Serum cortisol (nmol/L)	N/A	258.1 (81.2)	N/A	N/A
Salivary cortisol (nmol/L)				
Early morning; mean (sd)	19.7 (9.5)		22.9 (9.6)	21.3 (10.8)
Night time; median (IQR)	2.3 (1.5, 3.5)		2.4 (1.7,3.7)	N/A
Diurnal slope (nmol/L/h); mean (sd)	-1.13 (0.7)		-1.43 (0.7)	-4.3 (3.8)
Cortisol Awakening Response; mean (sd)	2.3 (9.4)		6.4 (11.7)	N/A
Exclusions due to being on corticosteroids; n(%)	62 (10%)	13 (1.2%)	19(1.1%)	396 (7.5)[a]

[a]endocrine medication

Table 2. Overall summary estimates of effect for the associations between cortisol measures and wellbeing from a series of meta-analyses.

	Included cohorts	Number of individuals	Mean difference in WEMWBS score (95% CI) per SD increase in cortisol							
			Model 1[a]				Model 2[b]			
			Regression coefficient (95% CI)	P-value	Tests of heterogeneity I^2(%)	P-value[c]	Regression coefficient (95% CI)	P-value	Tests of heterogeneity I^2(%)	P-value[c]
Early morning cortisol	All	7515	0.02 (-0.17,0.21)	0.8	2.3	0.4	0.01 (-0.22,0.24)	0.9	18.0	0.3
Evening cortisol	CaPS NSHD	1756	-0.47 (-1.00,0.05)	0.08	20.3	0.3	-0.31 (-0.83,0.21)	0.2	19.1	0.3
Flatter diurnal slope	CaPS NSHD NCDS	6490	-0.07 (-0.27,0.14)	0.5	0.0	0.6	-0.08 (-0.28,0.13)	0.5	0.0	0.6
Cortisol awakening response (CAR)	CaPS NSHD	1612	-0.19 (-0.62, 0.24)	0.4	0.0	1.0	-0.16 (-0.59,0.26)	0.4	0.0	0.9

[a]sex, age at cortisol assessment, follow-up time to wellbeing assessment; [b]Model 1 plus body mass index, smoking status, adult social class; [c]p-values from Cochran's Q statistic performed as a test of between-study heterogeneity

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Figure Legend

Figure 1. Meta-analysis of the association between wellbeing and early morning cortisol (sex and follow-up time adjusted)

Figure 2. Meta-analysis of the association between wellbeing and evening cortisol (sex and follow-up time adjusted)

Figure 3. Meta-analysis of the association between wellbeing and flatter diurnal slope (sex and follow-up time adjusted)

Figure 4. Meta-analysis of the association between wellbeing and cortisol awakening (sex and follow-up time adjusted)

Figure 1

276x190mm (300 x 300 DPI)

Cohort	N	Age range at cortisol measurement (y)	Regression coefficient (95% CI)	% weight
NSHD	1295	62-64y	-0.33 (-0.77, 0.11)	77.99
CaPs	461	65-83y	-0.98 (-2.03, 0.07)	22.01
Overall (I-squared = 20.3%, p = 0.263)			-0.47 (-1.00, 0.05)	100.00

NOTE: Weights are from random effects analysis

lower wellbeing   -2   -1   0   1   2   higher wellbeing

Regression coefficient per 1 sd increase in evening cortisol

Figure 2

137x74mm (300 x 300 DPI)

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Cohort	N	Age range at cortisol measurement (y)	Regression coefficient (95% CI)	% weight
NCDS	4318	44-45y	-0.09 (-0.33, 0.14)	77.06
NSHD	1154	62-64y	-0.06 (-0.53, 0.41)	19.53
Caps	416	65-83y	0.51 (-0.61, 1.63)	3.41
Overall (I-squared = 0.0%, p = 0.583)			-0.07 (-0.27, 0.14)	100.00

NOTE: Weights are from random effects analysis

lower wellbeing   -2   -1   0   1   2   higher wellbeing

Regression coefficient per 1 sd increase in diurnal slope (flatter slope)

Figure 3

508x381mm (300 x 300 DPI)

Figure 4

508x381mm (300 x 300 DPI)

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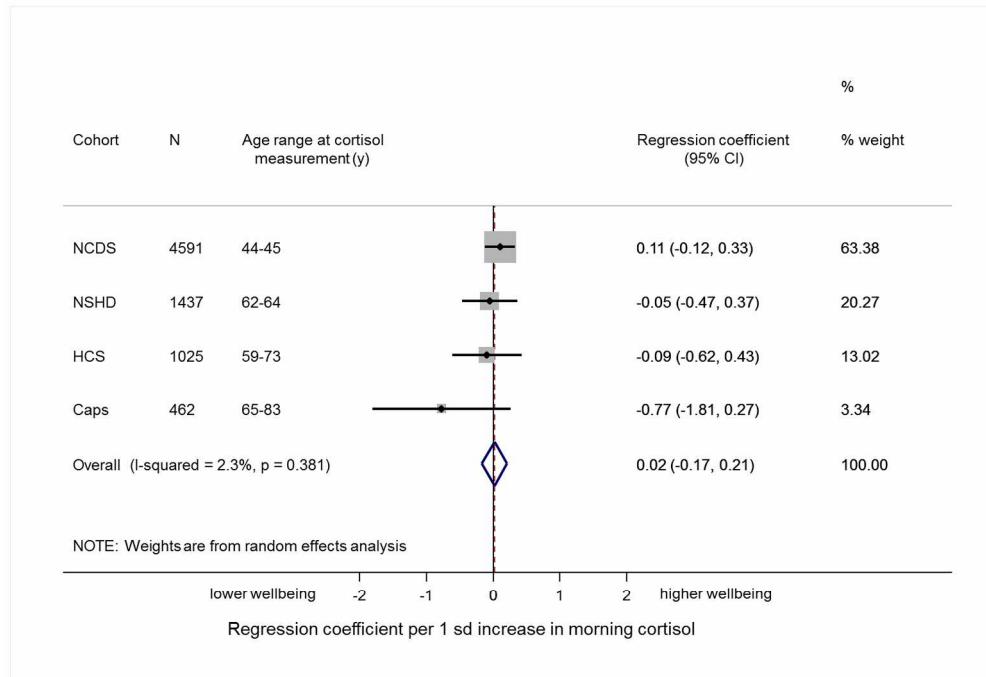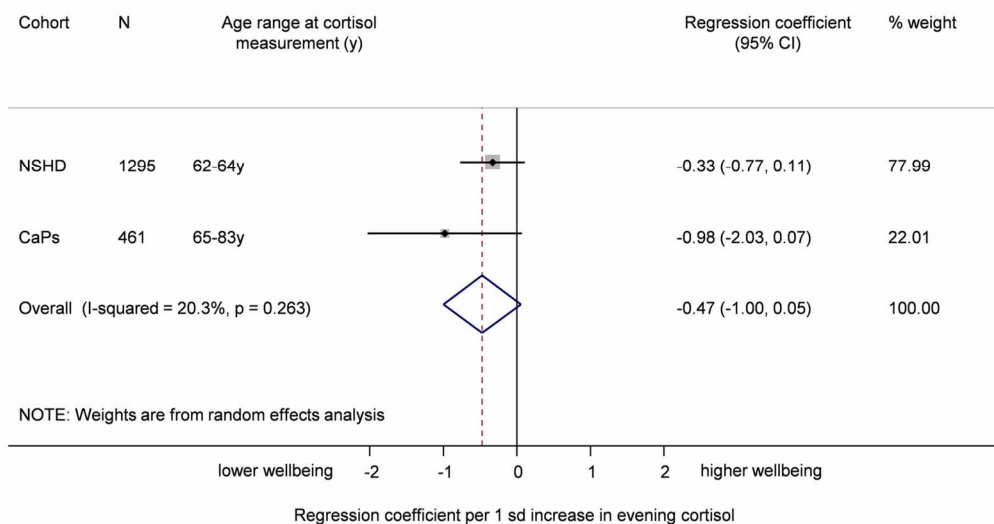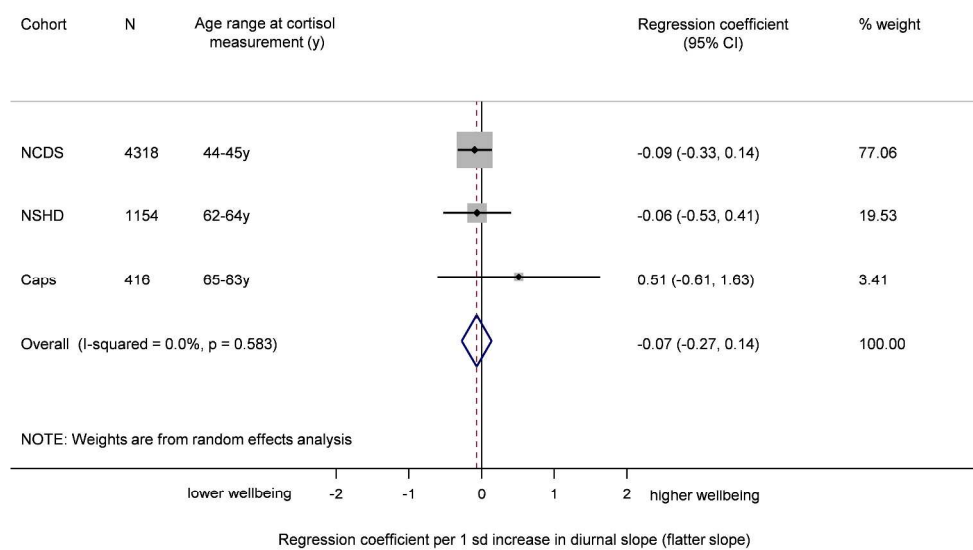STROBE Statement—checklist of items that should be included in reports of observational studies

	Recommendation	Page
Title and abstract	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1,2
	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1,2
Introduction		
Background/rationale	Explain the scientific background and rationale for the investigation being reported	4
Objectives	State specific objectives, including any prespecified hypotheses	5
Methods		
Study design	Present key elements of study design early in the paper	5
Setting	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5,6
Participants	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	5,6
	(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed	N/A
Variables	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5-7
Bias	Describe any efforts to address potential sources of bias	9-10
Study size	Explain how the study size was arrived at	9, Table 1
Quantitative variables	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	10
Statistical methods	(*a*) Describe all statistical methods, including those used to control for confounding	10-11
	(*b*) Describe any methods used to examine subgroups and interactions	11
	(*c*) Explain how missing data were addressed	11
	(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed	
	(*e*) Describe any sensitivity analyses	11

Continued on next page

Results		**Page**
Participants	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9, Table 1
	(b) Give reasons for non-participation at each stage	
	(c) Consider use of a flow diagram	N/A
Descriptive data	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
	(b) Indicate number of participants with missing data for each variable of interest	
	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	*Cohort study*—Report numbers of outcome events or summary measures over time	Table 1
Main results	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Table 2
	(*b*) Report category boundaries when continuous variables were categorized	
	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	12
Discussion		
Key results	Summarise key results with reference to study objectives	13
Limitations	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	14
Interpretation	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	16
Generalisability	Discuss the generalisability (external validity) of the study results	13-14
Other information		
Funding	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	17

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