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\*Corresponding author: Ru Jia, School of Medicine, University of Nottingham, University Park, Lifespan and Population Health Applied Health Research Building (No. 42), Nottingham NG7 2RD, UK.  
E-mail: [ru.jia@nottingham.ac.uk](mailto:ru.jia@nottingham.ac.uk)

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Daryl O'Connor, UK

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## HEALTH PSYCHOLOGY | REVIEW ARTICLE

# The Association of Lifestyle and Mood with Long-Term Levels of Cortisol: A Systematic Review

Ru Jia<sup>1\*</sup>, Sophie Carlisle<sup>1</sup> and Kavita Vedhara<sup>1</sup>

**Abstract:** Objectives: To critically review evidence for associations between long-term cortisol levels, mood, and lifestyle factors. Systematic searches of electronic databases (MEDLINE, EMBASE, PsycINFO, WoS, and CINAHL) were conducted up to 21/11/2020 to identify observational and interventional studies ( $n = 4971$ ) reporting associations between one or more lifestyle or mood factor with cortisol outcomes measured over  $\geq 4$  weeks in healthy adults. Quality of included studies was assessed using Downs and Black checklist. The quality of evidence supporting the associations of lifestyle and mood with long-term cortisol levels was assessed as being of moderate-to-poor quality. Observational studies ( $n = 25$ ) indicated positive associations for BMI/body weight ( $ESr$ , pooled effect size correlation = 0.15,  $p < .001$ ), physical activity ( $ESr = 0.16$ ,  $p < .001$ ), perceived stress ( $ESr = 0.114$ ,  $p = .02$ ), and depression ( $ESr = 0.133$ ,  $p = .02$ ), but not stressors ( $ESr = 0.06$ ,  $p = .29$ ), anxiety ( $ESr = 0.08$ ,  $p = .14$ ), or specific features of stress ( $ESr = 0.25$ ,  $p = .10$ ). There was insufficient evidence to reliably estimate associations between long-term cortisol levels and sleep, smoking, alcohol consumption, caffeine consumption, and PTSD. Findings from interventional studies ( $n = 27$ ) were mixed and did not always support the relationships found in observational studies. The findings of this review were limited by the quality of the evidence. Current evidence for associations between mood and

### ABOUT THE AUTHOR

The authors' research interests include experimental and applied research examining the relationship between psychological factors and health, disease, and treatment outcomes as well as the development of brief and complex interventions to improve these outcomes. The hormone cortisol is one of the key biological pathways by which psychological functioning can influence health and has, therefore, been the focus of much of the research of the group. This has included examining its role in influencing vaccine effectiveness and in vitro fertilisation outcomes, and most recently examining the role of cortisol in influencing the effects of mental health on physical health during the COVID-19 pandemic. (102 words)

### PUBLIC INTEREST STATEMENT

Cortisol is a hormone secreted by the adrenal glands. It plays a critical role in how we respond to stressful experiences and to behaviours, such as sleep, physical activity, diet, and smoking that can change during stress. In this review, we have brought together evidence from both observational studies and trials that have looked at the relationship between cortisol measured  $\geq 4$  weeks and mood and behaviours. Observational studies suggest greater weight, physical activity, perceived stress, and depression are associated with greater cortisol levels. The relationships between smoking and alcohol consumption and cortisol were inclusive because the evidence was insufficient. Findings from trials were mixed and did not always support the associations found in observational studies. Overall, the current evidence is mixed in quality and quantity. We recommend that future studies clearly describe why they measure cortisol, and directions of the associations between cortisol, mood, and behaviour they expect to find.

lifestyle factors with long-term levels of cortisol is mixed. For many factors, there was considerable uncertainty regarding the size of association with long-term cortisol due to a paucity of evidence. Future research should aim to (1) follow more consistent sampling protocols between studies and (2) clearly describe the hypothesised mechanisms through which interventions would affect cortisol levels.

**Subjects:** Endocrinology; Health Psychology; Stress in Adults

**Keywords:** Long-term Cortisol; Lifestyle; Mood; Systematic Review

### 1. Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is a critical pathway mediating the relationship between stress and health (Tsigos & Chrousos, 2002), and is among the most commonly investigated in the context of acute and chronic stress (McEwen & Wingfield, 2003). The final product of the HPA axis, cortisol, is a glucocorticoid steroid hormone secreted by the adrenal cortex, and is often characterised as a ‘stress hormone’ because of its critical role in the physiological response to psychological stress. However, when stressors occur, both physiological (e.g. hormone secretions) and behavioural (e.g. alcohol consumption and smoking) responses are triggered which (in the case of an adaptive response), contribute to the restoration and maintenance of homeostasis. For example, stress-related food consumption can contribute to changes in cortisol levels, which, in turn, impact glucose homeostasis and energy deposition (McEwen & Wingfield, 2003). Thus, cortisol is associated not only with mood, but also with a range of lifestyle factors that respond to and are affected by stress-related changes in homeostasis and circadian rhythms, e.g. sleep, physical activity, diet etc.

Several earlier reviews have examined the relationship between cortisol, mood, and lifestyle factors. However, these have been limited in a variety of ways, e.g. by focusing on one type of cortisol measure (e.g. hair cortisol); a specific cortisol index (e.g. diurnal cortisol slopes); or a specific population. For example, Adam et al. (2017) examined the association of diurnal cortisol slopes only with mental and physical health outcomes. For mental health outcomes, they found that flatter cortisol diurnal slopes were significantly associated with increased risk of depression, externalising disorders, and internalising disorders. In contrast, Lopresti and colleagues conducted a narrative review describing the relationship between cortisol and diet, sleep and exercise (Lopresti et al., 2013). For the diet, they found that diets high in fat and saturated fatty acids were associated with disturbed cortisol diurnal curves, while Omega-3 supplements were associated with lower or blunted cortisol activity. For sleep, they found evidence for higher cortisol levels in individuals with insomnia. For exercise, although some evidence suggests that acute exercise elevated acute levels of cortisol, the relationship between exercise in general and the HPA activity is complex and appears to be influenced by the type, duration, intensity, and chronicity of the exercise and the measure of HPA activity (Lopresti et al., 2013).

Although both reviews have helped enhance our understanding of the relationship between cortisol and mood and cortisol and lifestyle, their focus on a specific population and a limited number of indices of cortisol, mood and lifestyle necessarily limit conclusions. This is further compounded by the fact that both reviews focused on acute/short-term measures of cortisol. This, in part, reflects the history of research in this field, which has focused on short-term measures of cortisol (such as awakening cortisol, evening cortisol, or immediate cortisol levels after intervention or challenge). However, the pulsatile nature of the hormone (Young et al., 2004) and concerns regarding the health relevance of single isolated measures have increased interest in capturing levels over longer periods of time. (Adam et al., 2017)

This has been facilitated by innovations in the field and, in particular, the development of methods to assess cortisol in hair, with hair cortisol concentrations (HCC) being able to provide a

**Table 1. Summary of Studies Investigating Long-term Cortisol and Lifestyle Factors**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Chen et al. (2015/United States)	Observational	N/A	Caregivers of children with disabilities, n=85 (female=75, male=10)	Hair (HCC)	3 months	BMI (measured by researcher) Smoking (Self-identification of being smoker or not)	One-time assessment	HCC was not correlated with obesity status. No association between smoking status and HCC was found.
Faresjo et al. (2013/Sweden, Greece)	Observational	NA	General population, n=591 (female=321, male=270)	Hair (HCC)	3 months	Smoking (Self-identification of being daily smoker or not)	One-time assessment	No association between smoking status and HCC was found.
Garcia-Leon et al. (2018/Spain)	Observational	N/A	Pregnant women (n=62)	Hair (HCC)	3 trimester for pregnant participants	BMI (self-reported) Physical activity (self-reported regular/non-regular participant of physical activity)	One-time assessment	HCC was not correlated with BMI in pregnant women. Physical activity was significantly correlated with HCC in general sample.
Geng et al. (2016/China)	Observational	N/A	Participant were addicted to methamphetamine, n=51 (female=51)	Hair (HCC)	1 month	BMI (measure not described)	One-time assessment	No significant association between HCC and BMI was found.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Gerber, Kalak et al. (2013/ Switzerland)	Observational	N/A	General population, n=46 (female=26, male=20)	Hair (HCC)	3 months	Vigorous physical activity: assessed with an accelerometer worn around the hip. All activities with >5,724 counts/min were defined as vigorous.	7 consecutive days	Higher HCC was significantly associated with greater vigorous physical activity.
Heinze et al. (2016/UK)	Observational	N/A	n=30 were mental health help-seekers and n=28 were healthy controls, (female=52, male=6)	Hair (HCC)	6 months	BMI (measure not described) Smoking and alcohol consumption (Self-reported: Alcohol, Smoking and Substance Involvement Screening Test)	BMI: one-time assessment Smoking and alcohol consumption: over the past 3 months	No significant correlations between HCC and BMI. No significant associations between smoking and HCC either over the past 3 month or the 3 month before were found. No significant association between alcohol consumption and HCC over the past 6 month.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Larsen et al. (2016/Denmark)	Observational	N/A	Parents of children at risk of obesity, n=532 (female=301, male=231)	Hair (HCC)	1-2 months	BMI (measured by researcher)	One-time assessment	Higher HCC was significantly associated with higher BMI.
Manensijn et al. (2017/The Netherlands)	Observational	N/A	n=89 were shift workers and n=33 were controls, (m=122)	Hair (HCC)	3 months	BMI (measured by researcher)	One-time assessment	In shift workers there was a significant correlation between elevated levels of HCC and increased BMI.
Qi et al. (2014/China)	Observational	N/A	Healthy women, n=39	Hair (HCC)	1 month	BMI (self-reported)	One-time assessment	No significant correlations between HCC and BMI.
Sumra and Schillaci (2015/Canada)	Observational	N/A	Healthy women, n=31	Urine, hair (HCC)	1 month for urinary cortisol; 3 months for HCC	Physical activity: self-reported frequency	frequency of exercise per week	Neither hair, nor mean urinary cortisol was correlated with any other primary variables including physical activity (correlation coefficient not reported).

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Wang et al. (2016/United States)	Observational	N/A	Female veterans, n=82	Hair (HCC)	3 months	BMI (measured by researcher) Sleep quality (the Pittsburgh Sleep Quality Index)	BMI: One-time assessment Sleep quality: not described	No significant correlations between HCC and BMI. No significant relationship between self-reported sleep quality and HCC.
Batista et al. (2015/Brazil)	6-week Yoga intervention 50 minute sessions twice a week for 6 weeks. Postures included snake posture, boat posture, yoga mudra, Karmasana, self massage and deep relaxation.	Yoga practice would reduce stress, as reflected by cortisol	General population n=22 (female=15, male=7)	Saliva (daily rhythm of cortisol production, morning and night-time values)	Saliva samples were collected at 7.30am, 9am, noon, 12pm for 6 weeks (4 samples before and after yoga over 6 weeks).	NA	NA	Over long-term, Yoga induced higher cortisol production in the morning and lower production in the evening.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Brown et al. (2002/United States)	Diet intervention Crossover design of diet treatment for 11 menstrual cycles in total. Baseline + diet (polyunsaturated fat, PUFA) +washout + diet (soy protein + high saturated fat); control (high saturated fat). Each treatment lasting two menstrual cycles, with a washout time of two menstrual cycles between each treatment.	N/A	Healthy women n=26	Blood	11 menstrual cycles in total. Blood samples were collected for 2 menstrual cycles for each treatment. 2-day consecutive blood sample for mid-follicular phase (day 7&8 or 8&9) and mid-luteal phase (day 21&22 or 22&23) after menses during the second cycle of each diet treatment period and the baseline month.	Meals were provided to the subjects each day. The subject's free meal night food records were obtained each week during all diet treatments. Caloric intake was individualized for each subject. The standardised study diets were consumed with 37% of kilocalories as fat, 48% as carbohydrate, and 15% as protein. Only the type of fat and amount of soy protein were altered for the study diets.	NA	A high polyunsaturated fat diet may decrease cortisol concentrations by 81.8 nmol/l ( $p = 0.088$ ) compared with a high saturated fat diet. Compared with the control diet, no significant changes of cortisol were found for the soy diet.

(Continued)



**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Chen et al. (2017/China (Taiwan))	20-week Yoga intervention Prospective randomized control trial with a longitudinal, repeated-measures design. Intervention was yoga (70 minutes, twice per week for 20 weeks in groups of 10-12 women).	Yoga practice would reduce stress, as reflected by cortisol	Pregnant women n=101 (female=101)	Saliva	Saliva samples were collected 10 minutes before yoga and immediately after and at same time points for control group at 16, 20, 24, 28, 32 and 36 weeks gestational age (12 samples per participant)	NA	NA	No significant long-term effect of yoga on salivary cortisol levels.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Coiro et al. (2007/Italy)	8-week Alcohol abstinence Participants exercised on an electrically braked cycle ergometer. An initial load of 50 W was increased by 50 W every 3 minutes until subjective exhaustion, for about 15 minutes. Alcoholic patients were tested at 4, 6 and 8 weeks of alcohol abstinence.	Not described	Alcoholics n=20 (male=20) and healthy controls	Blood	Blood samples were taken at 30 minutes before exercise, initiation of exercise, 10, 20, 30, 40, 50, 60 minutes. Alcoholic participants provided 8 samples at 4, 6, 8 weeks after abstinence. Healthy controls were only assessed once.	Abstinence from alcohol was monitored with daily measurement of ethanol levels in 24-hour urine samples.	8 weeks	Serum cortisol levels significantly rose during exercise in the healthy controls and peaked after 30 minutes after the exercise task. However, the cortisol levels among abstinent alcohol patients did not significantly change during the exercise task, after 4-weeks of alcohol abstinence. At 8 weeks post-abstinence, the cortisol responses to exercise task for the abstinent alcoholic patients were significantly higher than 2 weeks previously and was no longer distinguishable from non-alcoholic participants.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Diment et al. (2012/UK)	8-week Supplemented diet intervention Normal (habitual) diet plus mixed nutritional supplement.		Healthy male soldiers n=30	Saliva	8 weeks Baseline, pre-field exercise at the end of week 6; post-field exercise at week 8.	A 24-hour dietary record from each participant on the day before their visits with study staff was acquired.	NA	No intervention effect on plasma cortisol was found.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Goldberg et al. (2014/Canada)	7-week mindfulness smoking cessation intervention: training or freedom from smoking enhanced. Participants were randomised to 1 of 2 smoking cessation interventions: mindfulness training for freedom from smoking enhanced. Both groups had 8 meetings (over 7 weeks) and made quit attempts 1 month after the 1 <sup>st</sup> meeting.	Negative affect and increased cortisol output under stress were related to increased risk of smoking relapse and that stress reduction interventions designed to cultivate emotion regulation skills and target social-emotional processes could aid smoking cessation and may be marked by lower levels of cortisol in hair.	Smokers n=135 (female=55)	Hair (HCC)	A 3cm hair sample was taken from each participant at 1-month post-quit study visit, to determine the HCC (1) during the month before the intervention, and (2) during the month post-quit	NA	NA	Participants' HCC was lower at 1-month post-quit compared to the month before the intervention. Intervention participants who stayed abstinent at the 1-month post-quit study visit had significantly lower levels of HCC compared with that in participants who had relapsed post-quit. However, differences were not found between two intervention groups for either decreases in HCC or the prevalence of smoking abstinence at post-quit.

(Continued)

Table 1. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Lovullo et al. (2005/United States)	4-week crossover caffeine stimulation intervention. On each week, participants were instructed to abstain for 5 days from dietary caffeine but took caffeine capsules, followed by a test day, and a crossover day to buffer sudden changes in intake between study weeks. On the test day, each participant was administered caffeine capsules three times and underwent a stress challenge between the first two administrations. In order to investigate the effect of different levels of caffeine intake on cortisol responses to caffeine challenges, three different maintenance doses were administered to participants, to mimic a range of consumption commonly found in the US diet: none (0 mg/day), moderate (300 mg/day), high (600 mg/day).	NA	General population n=96 (female=48, male=48)	Saliva	Eight saliva samples were collected from each participant on the test day each week, before and after the stress challenge and each administration of caffeine capsules.	NA	NA	Taking caffeine capsules on the test day after five days of abstinence resulted in a significant elevation of cortisol throughout the entire day. A significant elevation of cortisol was only seen in the group that had moderate caffeine intake of 300mg, but not 600mg, for five days. The elevation of cortisol in the 300mg group lasted only for about 6 hours on the test day.

(Continued)

**Table 1. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Mangine et al. (2017/United States)	8-week resistance training. Participants randomly assigned to high intensity low volume training or high volume moderate intensity training groups. Both groups required at least 28 training sessions in 8 weeks. Food intake was monitored via 3-day food diaries collected weekly.	Not described	Healthy resistance-trained men n=33	Blood (AUC)	Blood samples were collected at week 1 and 8, before exercise and immediately after exercise.	NA	NA	At week 1 of training, greater cortisol levels in response to exercise in the high-volume moderate-intensity training group were observed, compared to the high-intensity low-volume group. The difference in cortisol levels between groups was still significant at week 8 after training.

(Continued)

Table 1. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Miller et al. (2015/Australia)	5-week sleep restriction therapy Sleep restriction therapy comprised of one 40-minute face to face session which set a minimum time in bed and go to bed threshold time. Minimum time in bed and a 'go-to-bed' threshold time were set for each participant at the beginning of the intervention. All participants were instructed to not go to bed until after the threshold time and to only go to bed if they felt 'sleepy tired'. Up to 5 weekly phone calls were made to participants to review the minimum time in bed, which was modified weekly for each individual.	The sleep-restriction therapy would reduce early night plasma cortisol	People with insomnia n=11 (female=9, male=12)	Blood (mean plasma cortisol concentration)	Blood samples were taken intravenously every half hour from 22.00 to 06.00 on both nights of data collection at baseline and after the intervention.	Other sleep-related variables measured: Sleep onset latency; wake-time after sleep-onset; Insomnia Severity Index; Sleep Quality; Number of Awakenings; Total Sleep Time; Time in Bed	NA	Cortisol results from six patients showed that cortisol levels in the early morning were significantly higher post-intervention compared to baseline but no changes were observed in the pre-sleep phase or early part of the night at post-intervention.

(Continued)

Table 1. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Papacosta et al. (2013/Greece)	5-week exercise intervention Week 1 participants trained as normal. Weeks 2-3 participants trained at a higher intensity. Weeks 4-5 training was tapered to half of the normal training intensity (week 1 intensity).	Not described	Male judo athletes n=11	Saliva	2 saliva samples were collected at the end of each week during week 1-3, and everyday during week 4-5.	NA	NA	Evening cortisol fell below baseline at the beginning of the tapering week and then returned to baseline by the end of the taper training.
Sinha et al. (2011/United States)	Each participant who were part of a 1- month in-hospital abstinence intervention underwent the stress and alcohol cue tasks twice: at baseline and at 1-month-abstinence.	Not deescribed	Alcoholics n=72 (female=41, male=31)	Blood	Blood samples were taken from participants each time before and after the tasks, at baseline and follow-up at week 5.	NA	NA	Although there were no significant differences at baseline, alcoholic participants showed a lack of cortisol response to both stress and alcohol cues compared with non-alcoholic controls at 1-month-abstinence.
Tam et al. (2014/United States, Australia)	24-week exercise and Diet Intervention Randomised study with four groups: control (weight maintenance diet based on the American Heart Association Step 1 diet); calorie restriction 25%; calorie restriction 12.5% + exercise (supervised aerobic); low calorie diet until achieved 15% weight loss.		Overweight individuals n=46 (female=20, male=26)	Saliva (morning cortisol and diurnal cortisol) Morning cortisol was defined as the mean cortisol concentration at 08:00 and 08:30. Diurnal cortisol was calculated as the mean of the 8 cortisol measures across the day.	6 months (8 samples at baseline and end of intervention) Saliva samples collected at 8 time points over the day: 8:00, 8:30, 11:00, 11:30, 12:30, 1300;16:00 and 16:30.	Body composition (measured using dual x-ray absorptiometry and abdominal fat distribution by multi-slice computed tomography)	NA	At baseline and 24 weeks post caloric restriction, there were no associations between morning/diurnal cortisol levels and body composition (fat mass, visceral adipose tissue), in the whole group, or when groups were examined separately (data not shown).

(Continued)



Table 1. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Tanskanen et al. (2011/Finland)	7-week Exercise (military training) Participants divided into 3 categories according to levels of physical activity before military service, and then randomly selected 19 from each category.	Not described	Healthy male soldiers n=57	Blood	3 blood samples were taken at baseline and 3 at the end of intervention. Blood drawn from an antecubital vein after an overnight fast, 2 hours after breakfast, and immediately after exercise.	NA	NA	They reported that basal cortisol decreased from week 4 to week 7 of the training. Basal serum cortisol levels after 8 weeks of training were significantly higher in participants who were overreaching, compared to non-overreaching participants.
Timon et al. (2013/Spain)	8-week strength training program during 8 weeks with sessions 3 times a week.	Not described	Healthy women n=20	Urine	The first morning urine was collected from all subjects in three different moments across the menstrual cycle. Urine samples were taken the 1 <sup>st</sup> -2 <sup>nd</sup> day (menstruation phase), the 11 <sup>th</sup> -12 <sup>th</sup> day (follicular phase) and the 21 <sup>st</sup> -22 <sup>nd</sup> day (luteal phase). These samples were collected both the month before training and the month after training.	NA	NA	Significant changes in cortisol in urine following the training were not observed.

(Continued)

Table 1. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Lifestyle factors and measures	Time-frame lifestyle was measured	Results
Torres et al. (2008/Australia)	4-week Diet (crossover design: 2-week control and 4-week diet, followed by 2-week control and 4-week diet) (mood) Randomised cross over. Participants received two (of 3) 4-week diets (OD diet, high in potassium, magnesium and fibre and low in sodium and sat fat; LNAHK diet was lower in sodium and calcium; HC, high calcium), each preceded by a 2-week control diet. Subjects were instructed to follow the diets. During the CD and all test diet periods, a maximum of four Caffeine-containing drinks (e.g. cola drinks, coffee and tea) and two standard alcoholic drinks (10 g alcohol) were permitted daily.		General population n=94 (female=38, male=56)	Saliva (AUC)	10 weeks (3 samples at baseline and end of study) Saliva samples were collected at the end of each dietary period (4 weeks of diets, 2 weeks of control). A saliva sample was collected at approximately 1200, 1600 and 2000 hours			Higher cortisol levels were weakly associated with greater vigour, lower fatigue, and higher levels of urinary potassium and magnesium. However, the overall effects of interventions on cortisol were not reported.

**Table 2. Summary of Studies Investigating Long-term Cortisol and Mood**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Booij et al. (2015/The Netherlands)	Observational	N/A	Adult with and without depression (n=15/15), n=30 (female=22, male=8)	Saliva (daily slope of cortisol)	3 times a day (in the morning, six hours later in the afternoon, six hours later in the evening) for 30 days	Depression (the Beck Depression Inventory; depressed individuals were identified using the DSM-IV diagnose of Major Depressive Disorder)	Unclear	Cortisol in saliva was higher, and the daily cortisol slope steeper in the depressed participants compared to the non-depressed ones.
Braig et al. (2016/Germany)	Observational	N/A	Women shortly after delivery, n=768	Hair (HCC <sup>a</sup> )	3 months	Chronic stress (the screening scale of the Tirer Inventory of Chronic Stress) Anxiety (the Pregnancy-Related Anxiety Questionnaire; the Hospital Anxiety and Depression scale) Depression (the Hospital Anxiety and Depression scale)	Over the past 3 months	No association between chronic stress and HCC. No association between anxiety and HCC. No association between depression and HCC.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Byun et al. (2013/United States)	Observational	N/A	Caregivers of stroke survivors, n=63 (female=42, male=21)	Saliva (indices not specified)	Over 4 weeks (T1=within 2 weeks post-stroke, T2=6 weeks post-stroke)	Perceived stress (Perceived Stress Scale) Depression (the Patient Health Questionnaire)	Over the past month	Salivary cortisol on waking was not correlated with perceived stress but salivary cortisol in the evening had a positive correlation with higher levels of perceived stress. Salivary cortisol was not significantly correlated with depression at the beginning of the study but elevated cortisol levels in saliva in the evening were significantly correlated with greater self-reported depressive symptoms four weeks later.
Caparrros-Gonzalez et al. (2017/Spain)	Observational	N/A	Pregnant women, n=44	Hair (HCC)	9 months (over trimester 1, 2, and 3)	Depression (the Edinburgh Postnatal Depression Scale)	At postpartum but time-frame unclear	Higher HCC during 1 <sup>st</sup> and 3 <sup>rd</sup> trimester was prospectively associated with greater postpartum depression.
Chen et al. (2015/United States)	Observational	N/A	Caregivers of children with disabilities, n=85 (female=75, male=10)	Hair (HCC)	3 months	Perceived stress (Perceived Stress Scale)	Over the past month	No association between perceived stress and HCC.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Cieslak et al. (2011/United States)	Observational	N/A	People with PTSD, N=30 (F=17, M=13)	Saliva (AUC <sub>g</sub> , AUC <sub>i</sub> )	Saliva samples from participants four times a day (upon awakening, 1h, 4h, 12h after waking) at 1 week, 1 month, and 3 months after a traffic accident (i.e., trauma).	PTSD (the Impact of Event Scale)	At 1 week, 1 month, and 3 months after the accident	The impact of trauma at 1-month post-accident was negatively associated with AUC <sub>i</sub> (reflecting changes in daytime cortisol secretions) at 3 months and the impact of trauma at 3 months was positively associated with AUC <sub>g</sub> (reflecting total daytime cortisol release) at 1-month post-accident.
Faresjö et al. (2013/Sweden, Greece)	Observational	N/A	General population, n=591 (female=321, male=270)	Hair (HCC)	3 months	Perceived stress (Perceived Stress Scale) Anxiety (the Hospital Anxiety and Depression scale) Depression (the Hospital Anxiety and Depression scale)	Perceives stress: over the past month Anxiety and depression: unclear	No association between perceived stress and HCC. No association between anxiety and HCC. Higher HCC was significantly correlated with greater depression.

(Continued)

**Table 2. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Geng et al. (2016/China)	Observational	N/A	Participant were addicted to methamphetamine, n=51 (female=51)	Hair (HCC)	1 month	Perceived stress (Perceived Stress Scale) Implicit perceived stress (Implicit Association Test)	Over the past month	No association between self-reported perceived stress measured by Perceived Stress Scale and HCC. Higher HCC was significantly associated with greater implicit perceived stress.
Gerber et al. (2013/Switzerland)	Observational	N/A	General population, n=46 (female=26, male=20)	Hair (HCC)	3 months	Perceived stress (Perceived Stress Scale) Depression (the Beck Depression Inventory)	Perceived stress: over the past month Depression: unclear	Higher HCC was significantly correlated with lower perceived stress, but the correlation was weak. Lower HCC was significantly correlated with greater depression.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Giesbrecht et al. (2013/Canada)	Observational	N/A	Pregnant women, n=85	Saliva (Cortisol awaking levels, CAR, diurnal slope)	3 trimesters of pregnancy (saliva samples were collected 5 times per day over 2 consecutive days at approximately 14, 20, 32 weeks of gestation)	Distress (the Profile of Mood States)	At each sampling point over the same time frame.	Higher salivary cortisol was significantly associated with distress.
Grassi-Oliveira et al. (2012/Brazil)	Observational	N/A	Crack-cocaine-dependent women, n=23	Hair (HCC)	3 months	Mood impact of stressors (semi-structured interview with the Psykel Life Events Scale)	Over the past 90 days	Higher HCC was significantly correlated with the number of stressful events exposure in the previous 90 days and 30 days.
Heinze et al. (2016/UK)	Observational	N/A	n=30 were mental health help-seekers and n=28 were healthy controls, (female=52, male=6)	Hair (HCC)	6 months	Perceived stress (Perceived Stress Scale) Anxiety (the Overall Anxiety Severity and Impairment Scale) Depression (the Quick Inventory of Depression Symptom; the Kessler Psychological Distress Scale)	Perceive stress: Over the past month Anxiety: 3 months within the 6 months when cortisol was measured Depression: over the past 7-30 days.	No association between perceived stress and HCC. No association between anxiety and HCC. No association between depression and HCC.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Hoffman et al. (2017/United States)	Observational	N/A	Pregnant women, n=92	Hair (HCC)	3 trimesters (at 16, 28, 40 weeks of gestation)	Perceived stress (Perceived stress scale) Anxiety (the State-Trait Anxiety Inventory) Depression (the Center for Epidemiologic Studies – Depression Scale)	3 trimesters (at 16, 22, 28, 34, 40 weeks of gestation for the past month at each time)	Higher HCC was prospectively correlated with higher levels of perceived stress at 40 weeks of gestation. Higher HCC at 2-trimester was significantly correlated with perceived stress at 16 weeks of gestation. Greater anxiety measured at 16 and 28 weeks of pregnancy was correlated with higher HCC in both the second and the third trimester. Higher HCC during the 1 <sup>st</sup> trimester was prospectively associated with greater depression at 40 weeks of gestation, higher HCC during the 2 <sup>nd</sup> -trimester was significantly associated with greater depression at 16 and 28 weeks of gestation, higher HCC at the 3 <sup>rd</sup> -trimester was significantly associated with depression at 16 and 28 weeks of gestation.

(Continued)



**Table 2. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Kramer et al. (2009/Canada)	Observational	N/A	Pregnant women, n=651	Hair (HCC)	9 months	Perceived stress (Perceived Stress Scale) Anxiety (a 4-item scale) Depression (the Center for Epidemiologic Studies – Depression Scale)	Perceived stress: at 24-26 weeks of gestation for the past month. Anxiety: at 24-26 weeks of gestation Depression: at 24-26 weeks of gestation	No association between perceived stress and HCC. No association between anxiety and HCC. No association between depression and HCC.
O'Brien et al. (2013/United States)	Observational	N/A	General population, N=135 (F=88, M=47)	Hair (HCC)	3 months	Perceived stress (Perceived stress scale) Mood impact of stressors (Chaos in the neighbourhood in the past year, City Stress Index: chaos/order of the home environment, Chaos, Hubbub, and Order Scale)	Perceives stress: Over the past 3 months Mood impact of stressors: not specified	No association between perceived stress and HCC. No association between HCC and chaos either in the neighbours or in the home.
Qi et al. (2014/China)	Observational	N/A	Healthy women, n=39	Hair (HCC)	1 month	Work stress (the Effort-Reward Imbalance Scale)	Over the past month	Neither the effort nor the reward scores were correlated with HCC. However, the effort-reward imbalance (1.83*effort-score/reward-score) at work was positively correlated with HCC.

(Continued)

**Table 2. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Schalinski et al. (2015/Germany)	Observational	N/A	Women with stress-related disorders, n=45	Hair (HCC)	3 months	Depression (the clinical-administrated Hamilton Rating Scale) PTSD (the Clinician-Administered PTSD Scale)	Unclear	No association between depression and HCC. No association between PTSD and HCC.
Stoppelbein et al. (2015/United States)	Observational	N/A	Mothers of children diagnosed with cancer, n=27	Saliva (AUCg)	Saliva samples were collected between 3-5 pm on the first day, and between 6-8 am and 3-5 pm on the following day. This saliva collection protocol was repeated monthly for 12 months.	PTSD (the Posttraumatic Stress Disorder Checklist)	Over 12 months	Higher levels of cortisol were significantly associated with one type of self-reported PTSD symptoms: numbing symptoms
Sumra and Schillaci (2015/Canada)	Observational	N/A	Healthy women, n=31	Urine, hair (HCC)	1 month for urinary cortisol; 3 months for HCC	Perceived stress (perceived stress scale)	Over the past month	No association between perceived stress and HCC.
Ullmann et al. (2017/Germany)	Observational	N/A	General population, N=40 (F=22, M=18)	Hair (HCC)	3 months	Mood impact of stressors (mental burdening events, the Recalled Parental Rearing Behaviour Questionnaire)	Over the past 3 months	Those with mental burdening in the previous 3 months had higher cortisol compared with those without mentally burdening events.
Wang et al. (2016/United States)	Observational	N/A	Female veterans, n=82	Hair (HCC)	3 months	Depression (the Center for Epidemiologic Studies - Depression Scale)	Unclear	No association between depression and HCC.
Wikenius et al. (2016/Norway)	Observational	N/A	Pregnant women, n=181	Hair (HCC)	1-3 month (Second trimester)	Depression (the Edinburgh Postnatal Depression Scale)	Over the past week	No association between depression and HCC.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Bergen-Cico et al. (2014/United States)	4-week mindfulness intervention Weekly in person 90 minute mindfulness based meditation group sessions for 4 consecutive weeks. Participants were given a book and audio CD with guided practice.	Engagement in the stress-reduction intervention would reduce diurnal cortisol output and CAR among veterans with PTSD	Veterans n=40 (female=4, male=36)	Saliva(AUC <sub>g</sub> , AUC <sub>i</sub> , CAR)	Saliva samples were collected at waking, 45min after waking, 8hrs, 1.2hrs, and bedtime each day for 2 consecutive days, before treatment at baseline and after intervention.	Depression (Patient Health Questionnaire) PTSD (the Military PTSD checklist).	At baseline only	Significant changes in cortisol levels which was associated with engagement and number of sessions completed of the intervention. Participants who completed all four sessions had a significant decrease in the CAR and increase in AUC, compared with the control group. Changes in either PTSD or stress were not described.
Brown et al. (2016/United States)	8-week mindfulness intervention Participants attended weekly classes of 1.5-2 hours in length.	The intervention was to reduce psychological and neuroendocrine markers of stress.	Adult caregivers n=38 (female=32, male=6)	Saliva (diurnal cortisol)	Saliva samples were collected at 6 time point a day at 3 study periods (pre- and post-intervention, 3 month follow up).	Perceived stress (Perceived Stress Scale) Mood (Profile of Mood States)	NA	The mindfulness intervention significantly improved participants' perceived stress levels and mood. However, the improvements in mood, perceived stress, and burden among participants at 3-month follow-up were not significantly different between the mindfulness intervention and social support group. Neither conditions showed changes in diurnal cortisol response curve across the study time-frame.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Daubenmier et al. (2011/United States)	4-month mindfulness intervention. Twenty-four participants were asked to attend a weekly 2.5h class for nine weeks and a 7-hour silent day of guided meditation practice after class 6.	Mindfulness training would enhance awareness of and responsiveness to bodily sensations and reduce psychological stress; emotional eating, and cortisol secretion, which would lead to reductions in abdominal fat	Stressed women n=47	Blood; Saliva(CAR, cortisol slope)	Saliva sampled were collected upon awakening, 30 minutes after and before bed, on four days before and after the intervention. Blood samples were collected pre- and post-intervention.	Mindfulness (the Kentucky Inventory of Mindfulness Skills) Stress (Wheaton Chronic Stress Inventory; Perceived Stress Scale) Anxiety (The State-Trait Anxiety Scale)	NA	Participants in the intervention group showed significant improvements in mindfulness, anxiety and external-based eating after the intervention, compared to control participants. However, differences between the intervention and control groups were not significant for emotional eating, the average cortisol awakening response, overall weight, abdominal fat, or fat distribution over time. Neither groups showed significant changes in the cortisol slope or morning serum cortisol concentrations. Improvement of mindfulness, chronic stress, and Subgroup of obese participants in the intervention group showed significant reductions in the cortisol awakening response and maintained body weight while obese participants in the control group had a stable cortisol awakening response and gained weight.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Holt-Lundstad et al. (2008/United States)	4-week support enhancement intervention Twenty couples (n=40) in the intervention group were asked to attend two 1-hour group sessions for couple contact enhancement techniques. These couples were also instructed to practice the techniques for 30 minutes, 3 times per week for 4 weeks. A behaviour monitoring control group was designed where subjects were instructed not to change anything about their normal behaviours with their spouse and to simply keep a diary of their physical affection and mood.	Warm touch enhancement may improve mood and stress and result in reduced cortisol levels.	n=36 couples (female=36, male=36)	Saliva (AUC)	Saliva samples were collected five times throughout a day at pre- and post-intervention.	Unclear	Unclear	No significant intervention effect on salivary cortisol.

(Continued)

**Table 2. (Continued)**

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples (indices if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Huang et al. (2012/UK)	5-week stress reduction acupuncture intervention. The intervention group received weekly acupuncture sessions for 5 weeks. The relaxation-only group received weekly relaxation (without acupuncture) sessions for 5 weeks. The waitlist control group did not receive any treatment for 5 weeks.	Traditional Chinese acupuncture to treat chronic stress could change diurnal salivary cortisol profile in healthy individuals.	Stressed individuals n=18 (female=15, male=3)	Saliva (CAR, daily profile)	Saliva samples were collected four times a day on 9 days pre-, post-intervention and at follow-up after 2 weeks.	Perceived stress (Perceived Stress Scale)	NA	Perceived stress was reduced in all groups however the differences between pre- and post-intervention and between- and within-groups were not significant. Non-significant increased CAR was seen for both the intervention and relaxation-only groups. No significant changes in the decline in cortisol or the daily average cortisol concentrations were found between groups.
Jacobs et al. (2013/United States)	8-week mindfulness meditation intervention. The intervention group met twice per day for 1-hour sessions for meditation practice.	Whether self-reported mindfulness, as well as changes in mindfulness following the intervention, were related to resting cortisol. Direction of the intervention effect on cortisol not hypothesised.	General population n=60 (female=32, male=28)	Saliva (p.m. cortisol <sup>6</sup> )	Saliva samples were collected for 3 consecutive days at pre-retreat (2 weeks after arrival) and post-retreat (10 weeks after arrival). Samples were collected in the afternoon (2 hr after lunch) and at bedtime (right before sleep).	Self-reported mindfulness (the 37-item Five Factor Mindfulness Questionnaire)	NA	Self-reported mindfulness significantly increased after the intervention. No significant changes were found in cortisol levels. Changes in mindfulness were inversely associated with changes in resting p.m. cortisol levels.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Leivadi et al. (1999/United States)	5-week massage intervention. Participants randomised to a 30-minute session twice a week for 5 weeks. The relaxation control group received a 30-minute session twice a week for 5 weeks.	Massage therapy would improve mood, decrease cortisol and other stressors such as anxiety and neck, shoulder, and back pain.	General population n=30 (massage group=15, relaxation group=15)	Saliva	Saliva samples were collected twice a day pre- and post-intervention.	Anxiety (State Anxiety Inventory) Mood (Profile Of Mood States)	NA	Both groups reported improvements in mood and anxiety after sessions. Changes in mood, anxiety, or cortisol levels were not observed over time. Participants reported reduced n neck, shoulder, and back pain after the treatment sessions.F.
Limm et al. (2011/Germany)	One-year work-site stress-management intervention. The intervention consisted of two full-day group sessions followed by two refresher sessions within the next 8 months. At each group session, participants were asked to remember and share individual stressful situations at work. Several tools to cope with difficult and stressful situations were introduced by the trainer at the group session.	Hypothesised intervention effects on cortisol not described.	General population n=174 (female=22, male=152)	Saliva	Saliva samples to measure cortisol were collected seven times throughout a day both at pre-intervention and at 1-year.	Perceived stress reactivity (Stress Reactivity Scale) Effort-reward imbalance (the Effort-Reward Imbalance Scale) depression (the Hospital Anxiety and Depression Scale)	NA	Perceived stress reactivity reduced in both groups but a significantly greater reduction was seen in the intervention group at 1-year follow-up. No intervention effect on cortisol was observed. Improvements were seen for anxiety, depression, effort-reward imbalance and while greater in the intervention group compared to the control group, they were not significant.

(Continued)

Table 2. (Continued)

Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Lynch et al. (2011/UK)	8-week mindfulness-based coping intervention Participants attended one 1.5h meeting per week for eight consecutive weeks.	Whether the intervention reduce stress which may be reflected by the biomarkers of stress. Direction of the intervention effect on cortisol not hypothesised.	General population n=60	Saliva	Saliva samples were collected 8 times throughout the day for 2 consecutive days at pre- and post-intervention.	Mindfulness (Five Factor Mindfulness Questionnaire) Perceived stress (Perceived Stress Scale) Anxiety and depression (the Hospital Anxiety and Depression Scale)	NA	Mindfulness intervention significantly improved perceived stress, anxiety, depression among participants, along with increased self-reported mindfulness. However, cortisol levels did not significantly change after the intervention.
Marcus et al. (2003/United States)	8-week mindfulness-based stress reduction intervention Participants attended a mindfulness meditation session for 2.5 hours per week for eight consecutive weeks. They were also asked to practice mindfulness outside the sessions for 45-60 minutes for 6 days per week.	Whether the intervention reduce stress reflected by the biomarkers of stress. Direction of the intervention effect on cortisol not hypothesised.	People in therapeutic community n=21 (female=18, male=3)	Saliva (AUC)	Saliva samples were collected at 0, 30, 45 and 60 minutes after awakening on the first morning pre intervention and post intervention.	Perceived stress (Perceived Stress Scale)	NA	A non-significant decrease in self-reported perceived stress after intervention among participants was found. The awakening salivary cortisol levels were significantly lower following the intervention.

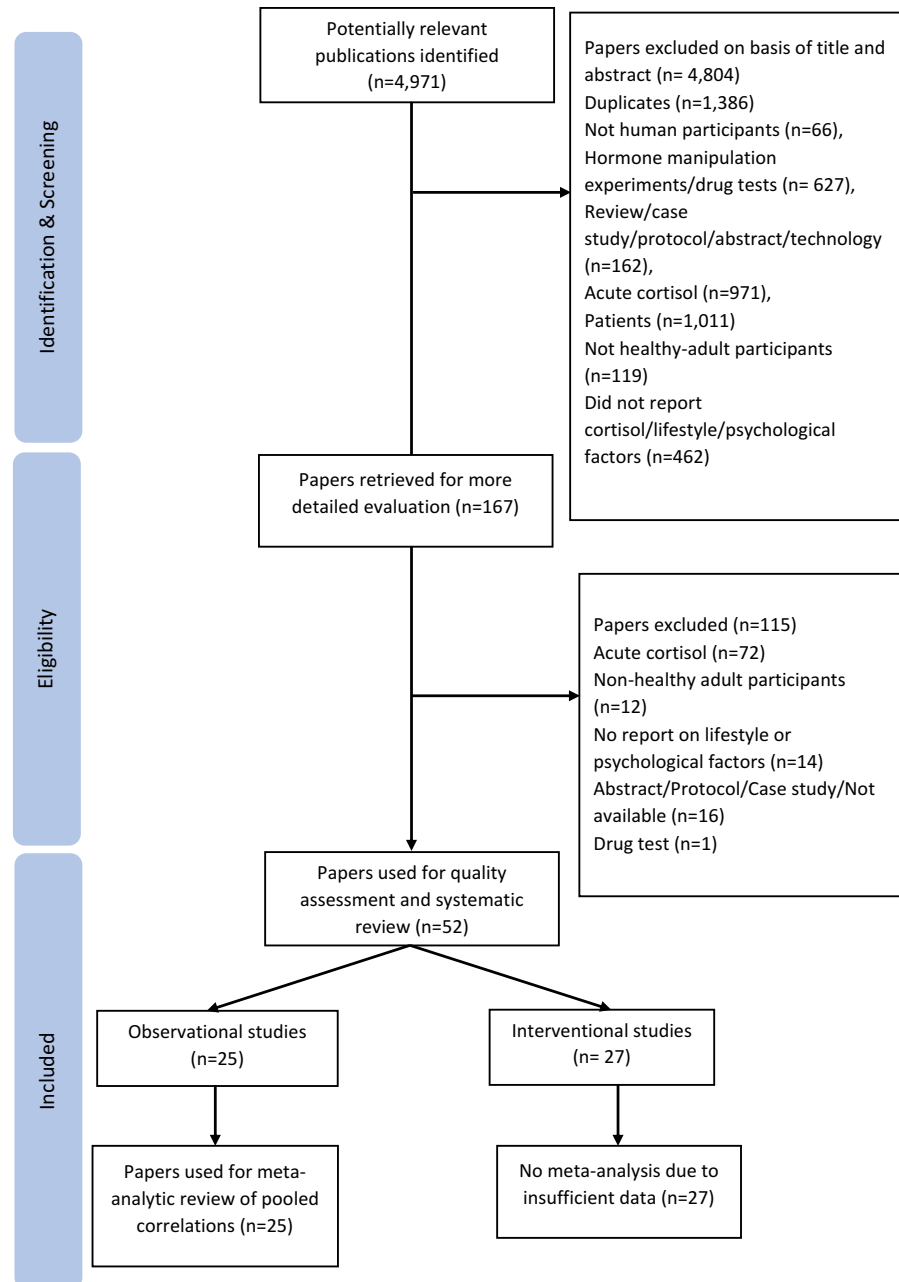
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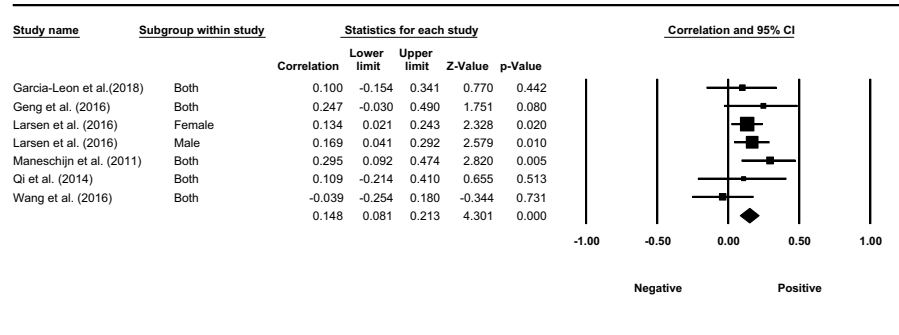
Author (Year/Region)	Study type	Hypothesised intervention mechanism	Population and Study Size	Type of samples if specified)	Time-frame cortisol was measured	Mood factors and measures	Time-frame mood was measured	Results
Sannes et al. (2015/United States)	10-week cognitive behavioural stress management. The intervention involved 10 two-hour sessions in groups of 4-10 on a weekly basis, which included education on awareness of the effects of stress, cognitive reframing, cognitive coping skills training etc.	Women in the intervention group would demonstrate lower intraindividual cortisol variability (i.e., the degree to which an individual's cortisol output may be erratic on a given day).	Healthy women at risk of breast cancer n=91	Saliva (cortisol slope, AUCg, CAR, individual cortisol variability)	Saliva samples were collected 5 times a day for 2 consecutive days at pre- and post-intervention, and at 1-month follow-up.	Unclear	Unclear	Lower levels of intraindividual cortisol variability among intervention participants were found, compared to the waitlist control group. Improvements towards a steeper cortisol slope and less overall cortisol output (area under the curve with respect to ground, AUCg) were also observed in the intervention group. Significant changes in other cortisol indices were not found.
Tsiouli et al. (2014/Greece)	8-week stress management Intervention group were trained in progressive muscle relaxation and diaphragmatic breathing for 37 minutes. They were asked to perform these techniques twice a day for 8 weeks, and asked to adapt a healthy lifestyle.	The intervention could reduce perceived and parenting stress, increase internal locus of control, promote healthy lifestyle, and normalise cortisol levels. However the direction of changes in cortisol was not specified.	Caregivers of children and adolescents with type 1 diabetes n=80	Saliva (diurnal cortisol)	Samples collected at 5 time points throughout a day pre- and post-intervention.	Lifestyle (questionnaire including quality of sleep, eating habit, smoking habits, drug use, physical activity etc.) Perceived stress (Perceived Stress Scale) Parental Stress (Parenting Stress Index-Short Form)	NA	No statistically significant improvement was found in cortisol levels in either the intervention or the control group. Stress was significantly reduced in the intervention group. Improvement in lifestyle factors such as sleep, stress-related symptoms, and eat habits were improved in both groups.

**Figure 1. PRISMA flow diagram of study selection.**

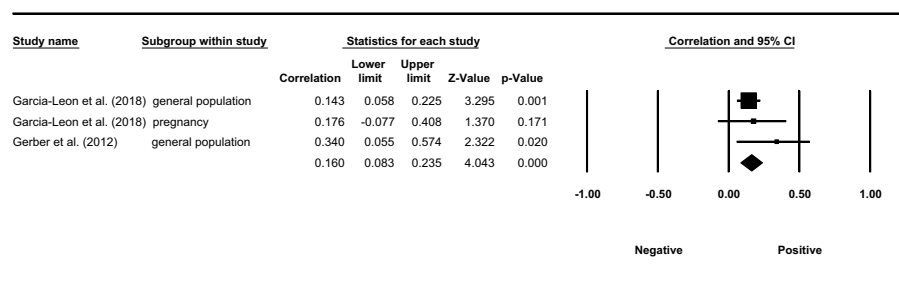


description of cortisol levels over periods of one to 6 months (Stalder & Kirschbaum, 2012). Wosu et al. (2013) and Stalder, Steudte-Schmiedgen et al. (2017) conducted separate systematic reviews to investigate correlates of HCC. Wosu et al. (2013) examined the association between HCC and socioeconomic factors (e.g. income, education, etc.), occupational stressors (e.g. shift work), age, sex, ethnicity, psychiatric symptoms and disorders (e.g. bipolar disorder and PTSD), adrenocortical conditions (e.g. Cushing syndrome), pregnancy, early life adversity (e.g. childhood maltreatment), BMI, waist-to-hip ratio, alcohol use, cigarette smoking, oral contraceptive use, and physical activity (Wosu et al., 2013). Focusing on their findings of direct interest in this review (i.e. indices of mood and lifestyle) they found associations between HCC and psychiatric symptoms and disorders, occupational stressors, physical activity, BMI, waist-to-hip ratio, and alcohol intake. They also reported that the available evidence did not support an association between HCC and cigarette

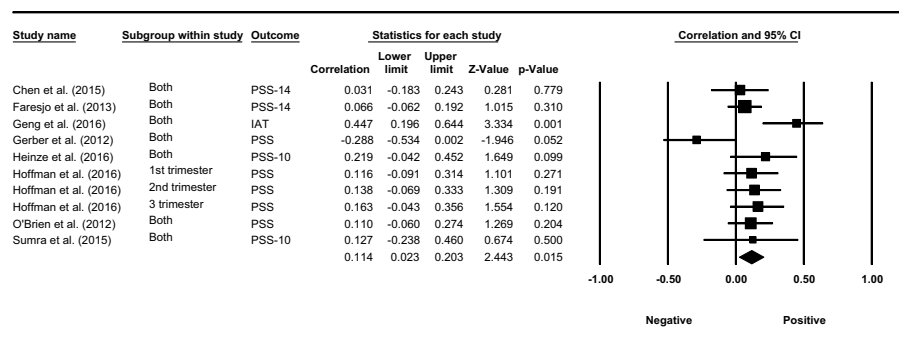
**Figure 2. Random effect meta-analysis for the correlation between long-term levels of cortisol and BMI.**



**Figure 3. Random effect meta-analysis for the correlation between long-term levels of cortisol and physical activity.**



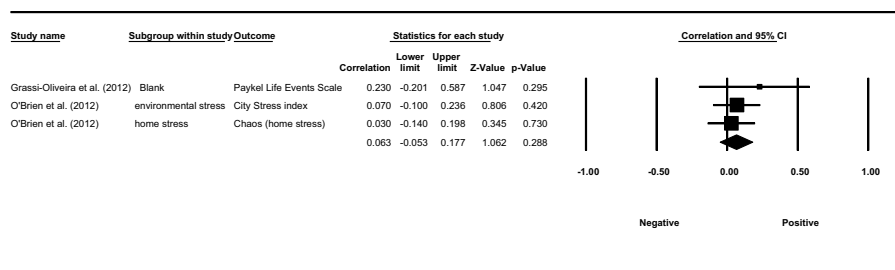
**Figure 4. Random effect meta-analysis for the correlation between long-term levels of cortisol and perceived stress.**



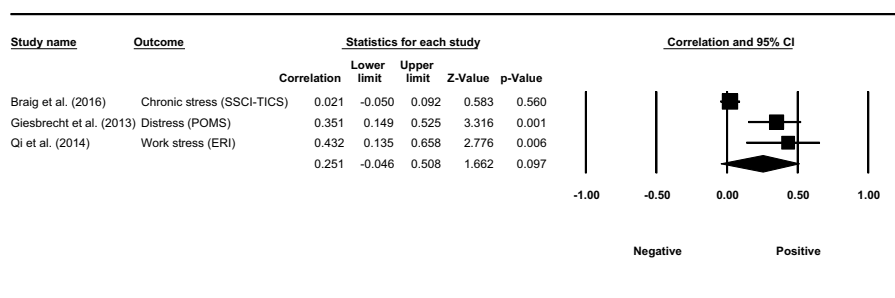
smoking (Wosu et al., 2013). They also did not find sufficient evidence to examine the association between diet, sleep quality, caffeine consumption, and HCC.

However, while comprehensive, the Wuso et al. review only narratively synthesised evidence on possible correlates of HCC. In contrast, Stalder, Steudte-Schmiedgen et al. (2017) quantitatively investigated the correlation between HCC and age, sex, BMI, waist-to-hip ratio, hair washing frequency, hair treatment, smoking, oral contraceptive use, blood pressure, previous and current chronic stressors, social support, and depression symptoms. Again focusing on mood and lifestyle indices alone, their meta-analysis showed that HCC was significant and positively associated with previous and current chronic stress, BMI, waist-to-hip ratio, blood pressure, and negatively associated with anxiety disorders. However, no significant associations were found between HCC and other mood disorders, social support, or smoking.

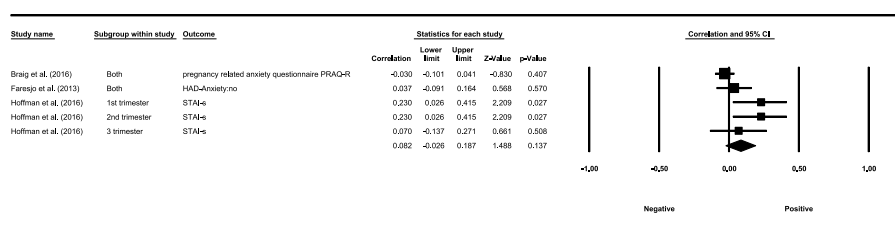
**Figure 5. Random effect meta-analysis for the correlation between long-term levels of cortisol and mood impact of stressors.**



**Figure 6. Random effect meta-analysis for the correlation between long-term levels of cortisol and other indices of stress.**



**Figure 7. Random effect meta-analysis for the correlation between long-term levels of cortisol and anxiety.**



In sum, previous reviews have been limited by their focus on a restricted range of indices of cortisol, mood, and/or lifestyle factors and none, to date, have included evidence from intervention studies. Thus, we sought in this review to address these gaps and provide an updated synthesis of the evidence pertaining to the nature and direction of the associations between long-term levels of cortisol, lifestyle, and mood factors.

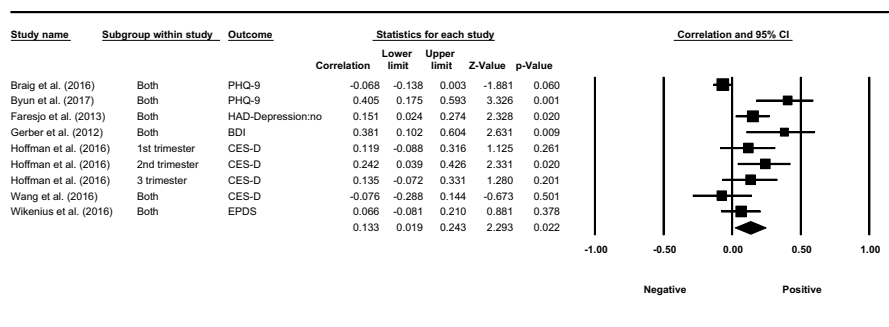
## 2. Method

### 2.1. Eligibility criteria

#### 2.1.1. Population

This review focussed on healthy adults aged 18–65 years with no medical condition or mental disorder and with no medication intake. This was because conditions, such as Cushing’s syndrome major depressive disorder, and use of certain medications (e.g. steroids), are associated with changes in cortisol levels (Casals & Hanzu, 2020; Rothe et al., 2020), which may confound the relationship between lifestyle/mood and long-term cortisol. Patients undergoing any type of medical treatment were excluded for similar reasons.

**Figure 8. Random effect meta-analysis for the correlation between long-term levels of cortisol and depression.**



### 2.1.2. Cortisol measures

This review focused on long-term levels of cortisol only. This included hair cortisol and longitudinal changes in cortisol measured in either blood, saliva, or urine. Hair cortisol is usually measured in 1–3 cm hair segments representing cortisol in the past 1–3 months Stalder & Kirschbaum, 2012). Although other cortisol indices, such as cortisol reactivity, CAR, AUCg, and diurnal slope are typically considered as acute cortisol indices (Saxbe, 2008), studies that investigated longitudinal changes of such cortisol indices were included in this review. The minimum time frame for studies measuring cortisol in hair is 1 month (4 weeks). Therefore, to assess evidence that could be considered to be comparable, repeated measurements of cortisol using any of the aforementioned acute measures (i.e. blood, saliva, urine, and follicular fluid; Stalder & Kirschbaum, 2012) had to cover a minimum period of at least 4 weeks to be included in this review. Studies involving the manipulation of cortisol levels through pharmacological agents were excluded.

### 2.1.3. Intervention

Interventional studies targeting lifestyle factors (physical activity, diet, caffeine consumption, alcohol consumption, smoking, and sleep), surrogates for lifestyle (e.g. BMI), and measures of mood (e.g. stress, anxiety, depression, and PTSD) were included.

### 2.1.4. Outcomes

Reported associations between long-term levels of cortisol and lifestyle and/or mood factors served as outcomes. In interventional studies where acute levels of cortisol were measured repeatedly, reported changes in cortisol levels at different time points within and between groups were also regarded as outcome variables. Comparisons of cortisol indices between patients and healthy participants were excluded, where relationships between lifestyle factors and cortisol indices were not measured and reported. Intervention effects on both lifestyle/mood and cortisol were also reported as outcomes for interventional studies. For studies reporting associations between cortisol and multiple lifestyle or mood factors, each association was included based on the lifestyle or mood factors reported. However, if a study reported an association between cortisol and combined lifestyle or mood factors as the outcome, these outcomes were removed due to difficulties inherent in separating the influence of different parameters.

### 2.1.5. Study design

Both observational and interventional studies are included in this systematic review. To meet the inclusion criteria, interventional studies had to show evidence of changes in at least one lifestyle factor, including BMI, physical activity, diet, caffeine consumption, alcohol consumption, smoking, and sleep or at least one mood factor, including stress, anxiety, depression, PTSD. Non-interventional studies had to investigate the relationship between any of the aforementioned lifestyle or mood factors and cortisol.

## **2.2. Exclusion criteria**

Reviews and conference abstracts were excluded from the review. Editorials, newspaper articles, and other forms of popular media were excluded. Case studies and qualitative studies were excluded as well. Non-English papers were excluded. Failure to meet any one of the above eligibility criteria resulted in exclusion from the review. A number of excluded studies and reasons for exclusion for those excluded following a review of the full text were recorded at each stage.

## **2.3. Information search**

The systematic review was registered with PROSPERO (No. CRD42019112339) and the protocol can be accessed here. Searches were conducted in the following electronic databases to identify relevant articles: MEDLINE, EMBASE, PsycINFO, Web of Science, and CINAHL. The initial search was conducted on 23 January 2018 and updated on 22 October 2018 which to identify one recently published study. Another update was conducted on 21 November 2020 which did not identify any eligible studies. Reference lists of eligible papers were also searched to locate additional studies that were not identified by the database search but may be eligible for the review.

Medical Subject Heading (MeSH) terms combined with free-text searches were used and adjusted for each database as necessary. The search was limited to full text and English articles. The title and abstract of each article were checked manually against the inclusion/exclusion criteria to exclude irrelevant articles or studies that did not fit the objectives of this review. Given the large number of results returned from the initial search, title, and abstract screening was conducted by one reviewer (RJ) only. Full-text screening and assessment were conducted by two reviewers (RJ and SC) with strong inter-rater reliability (Cohen's kappa = 0.83) on inclusion/exclusion. Disagreement on inclusion or exclusion for 6 out of 157 studies were resolved via discussion.

## **2.4. Assessment of risk of bias**

Risk of bias was assessed using the 27-item Downs and Black checklist (Downs & Black, 1998) which was identified as being suitable for use in systematic reviews of both trials and non-trials (Babu et al., 2016; Deeks et al., 2003; Donnelly et al., 2016). The checklist assesses five domains of the study reporting and design: reporting, external validity, internal validity (bias), internal validity (confounding), and power. Item 27 on the checklist was modified to suit the studies assessed (Huffer et al., 2017). For each item, a score of 1 was given if it answers yes and 0 if it answered no or was unable to determine an item. Not applicable (N/A) was recorded on the checklist where the item did not correspond well with the study design. The assessment of the risk of bias was conducted by two reviewers (RJ and SC) independently with 93% agreement on the items. Discrepancies were recorded and discussed between reviewers (RJ and SC) until agreements were achieved. Categorisation of the total risk of bias scores was adapted from Huffer et al. (2017) and is presented in Supplementary Appendix Table 3.

## **2.5. Data extraction and meta-analysis**

Data extraction was conducted independently by two reviewers (RJ and SC). Data extraction for each study included information on publication year, country or region where the study was conducted, sample size, age of study sample, population description, design of the study (observational or interventional), protocol for measuring cortisol (type of cortisol, cortisol indices calculated, time period measured, times of samples, number of samples per day).

Due to the high heterogeneity in the designs of intervention studies (e.g. type of lifestyle, length of follow-up, and type of cortisol measure) meta-analyses were conducted only for observational studies. Meta-analyses using random-effects models were conducted to determine the correlation between long-term levels of cortisol and BMI, physical activity, perceived stress, anxiety, and depression, where data were sufficient and available. Effect sizes were expressed as Pearson's  $r$  for all studies. When  $r$  values were available from the study they were entered into the meta-analysis. Otherwise, the  $r$  values were calculated from other statistics available (e.g.  $p$ -values and sample sizes). When long-term cortisol levels were measured at multiple points in a study (e.g.

different trimesters of pregnancy), reported correlations at each time point were treated as independent. When the correlation of cortisol with lifestyle or mood factors was reported separately for subgroups (e.g. men and women), results for each group were treated as independent. Comprehensive Meta-analysis Software (Version 13.0) was used to calculate the pooled correlations (ESrs).

### 3. Results

#### 3.1. Study characteristics

The database search resulted in 4,971 full titles. By 21 November 2020 a total of 52 publications including 25 observational and 27 interventional studies were included in the review and results reported after title and abstract screening and full-text assessment. The flow diagram of the screening and assessment process is presented in [Figure 1](#). Studies included in the review were conducted between 1999 and 2018, across 16 countries including the US, Brazil, China, and several European countries. Sample sizes ranged from  $n = 23$  to  $n = 768$  (median = 164) for observational studies and from  $n = 10$  to  $n = 135$  (median = 30) for interventions. [Table 1](#) and [Table 2](#) present summaries of the studies.

##### 3.1.1. BMI and body weight

Eight observational studies examined the association between BMI or body weight and long-term cortisol levels in hair. Six studies did not find a significant association between BMI and HCC whilst two studies found that HCC was positively associated with higher BMI. A meta-analysis ([Figure 2](#)), which included available data from six of these eight studies showed a positive and significant pooled correlation between BMI and HCC (ESr, pooled effect size correlation = 0.148,  $p < 0.001$ , CI = [0.213, 4.301]). However, the two studies that were not included in the meta-analysis for insufficient data both reported null association of HCC and BMI ( $n = 85$ , X. Chen et al. (2015),  $n = 58$ , Heinze et al. (2016)).

One intervention examined whether a 24-week moderate caloric restriction by diet alone or with an aerobic exercise intervention could result in decreased salivary cortisol levels (Tam et al., 2014). They found significant weight loss in the diet groups (both with and without exercise) and significantly higher morning and diurnal cortisol levels at week 24 compared to baseline in all groups. However, group, time, and sex effects on cortisol levels were not found.

In summary, although only two out of the eight observational studies reported a significant positive association between cortisol measured in hair and BMI, this observation was supported by the results of the meta-analysis. However, the intervention showed that although a 25% calorie restriction diet along or with exercise contributed to significant weight loss, it did not induce changes in cortisol levels.

##### 3.1.2. Diets

No observational studies examined the relationship between diet and cortisol levels. Three studies examined the effects of dietary interventions on long-term levels of cortisol, among which two measured cortisol in the blood and one in saliva. The two studies did not specify the hypothesised intervention effects on cortisol. B. D. Brown et al. (2002) compared the effects of cortisol measured in the blood of a high saturated fat Western control diet with two other diets: the control diet plus soy protein (soy diet) and the control diet with polyunsaturated fat (PUFA diet) in women. They found that compared with the control diet, PUFA diet induced a decrease in cortisol, while no significant changes in cortisol measured in blood were found for the soy diet. Torres et al. (2008) compared the effects of salivary cortisol on a low-sodium, high-potassium diet and a high-calcium diet with a moderate-sodium, high-potassium, high-calcium diet. The study reported that higher cortisol levels were positively associated with greater vigour, lower fatigue, and higher levels of urinary potassium and magnesium. Diment et al. (2012) hypothesised that participants who underwent a daily nutritional supplement intervention would have decreased plasma cortisol



levels at post-exercise compared to baseline. However, this hypothesised intervention effect was not found. Taken together, the effects of these dietary interventions on long-term levels of cortisol seem to be null. However, due to the high heterogeneity of these studies, definitive conclusions cannot be drawn.

### 3.1.3. *Physical activity*

Three observational studies examined the association between physical activity and HCC, with HCC measured over 3 months in all three studies. However, none of the studies measured physical activity for 3 months (i.e. the same period as cortisol). Two studies measured physical activity in participants for a typical week, among which one measured physical activity levels using accelerometers, and found a correlation between greater physical activity and greater HCC, and the other assessed self-reported frequency of exercise per week and did not find significant association between physical activity and HCC. Another study found that participants who were self-reported regular exercisers in general had higher HCC, although the same result was found in a subgroup of pregnant women. Meta-analysis of the three studies (Garcia-Leon et al., 2018; Gerber et al., 2013; Sumra & Schillaci, 2015) showed a significant positive pooled correlation between physical activity and HCC ( $r = 0.160$ ,  $p < 0.001$ ,  $CI = [0.235, 4.043]$ , Figure 3).

Six interventions examined the effects of physical activity or exercise on long-term levels of cortisol measured in blood, saliva, and urine, including two yoga interventions and four exercise/training programmes. Only one of these studies hypothesised the direction of intervention effects on cortisol (P. J. Chen et al., 2017). Two studies examined the effect of yoga on salivary cortisol through reducing stress, among which one found reduced perceived stress post-intervention and reported that cortisol levels in saliva were higher in the morning and decreased during the day, reaching lower values before sleep, compared to baseline where participants had an increase in cortisol before sleep (Batista et al., 2015). Another study did not find a significant effect of yoga on long-term salivary cortisol levels but reported higher levels of long-term cortisol in the intervention group (P. J. Chen et al., 2017). Four studies examined the effects of different training programs on cortisol. Mangine et al. (2017) found that greater cortisol levels measured in blood in response to exercise in the high-volume moderate-intensity training group were observed at week 8 after training, compared to the high-intensity low-volume group. Papacosta et al. (2013) observed that evening salivary cortisol fell below baseline at the beginning of the tapering (i.e. gradual reduction in the training load) week and then returned to baseline by the end of the taper training. Tanskanen et al. (2011) reported decreased basal serum cortisol levels from week 4 to week 7 of the training and significantly higher basal serum cortisol levels in participants who were over-reaching after 8 weeks of training. Timon et al. (2013) did not observe any significant changes in cortisol in urine following a strength training intervention across the menstrual cycle in women.

The observational studies suggested that physical activity is likely to be positively associated with cortisol measured in hair, which was somewhat supported by results of interventions, with four out of six trials reporting significant changes in cortisol levels after physical activity interventions. However, the types of physical activity and intervention effects on cortisol were all different among these studies. Therefore, we conclude that although physical activity is likely to be associated with cortisol levels, the direction of the relationship remains unclear.

### 3.1.4. *Sleep*

There is currently insufficient evidence to conclude the association between sleep and long-term levels of cortisol from both observational and interventional studies. Only one observational study investigated the relationship between HCC over 3 months and self-reported sleep over an unclear time frame and no significant relationship between self-reported sleep quality and HCC was found (Wang et al., 2016). Only one intervention examined whether a sleep restriction intervention would reduce early night plasma cortisol (Miller et al., 2015). They found that eight out of eleven insomniacs who completed the intervention showed decreases in insomnia severity after the



treatment. Contrary to their hypothesis, however, results from six patients showed that cortisol levels in the early morning were significantly higher post-intervention compared to baseline.

### 3.1.5. *Smoking*

Three observational studies examined the association between smoking and HCC and none of them found a significant association. Of the three studies, two measured HCC over 3 months and asked participants whether they were smokers. One other study measured HCC over 3–6 months and measured smoking and smoking-related problems in the past 3 months using the Alcohol, Smoking and Substance Involvement Screening Test. Meta-analysis of the association between smoking and HCC was not conducted due to insufficient data.

Only one trial assessed the effect of smoking cessation intervention on cortisol levels measured in hair. Goldberg et al. (2014) found that participants' HCC was lower at 1-month post-quit compared to the month before the mindfulness-based intervention and that those who stayed abstinent at the 1-month post-quit had significantly lower levels of HCC compared with those in participants who had relapsed. Differences in HCC and smoking cessation between the two intervention groups were not found. Participation in mindfulness training and smoking cessation itself was found to be independently associated with reductions in hair cortisol.

Evidence from observational and interventional studies seemed contradictory: none of the three observational studies reported a significant association between smoking and HCC, but the intervention found reduced HCC among participants of a smoking cessation intervention.

### 3.1.6. *Alcohol consumption*

Only one observational study investigated the association between alcohol consumption and HCC and reported that there was no significant correlation between self-reported use of alcohol and HCC measured over 6 months. (Heinze et al., 2016). With insufficient evidence, the association between alcohol consumption and long-term levels of cortisol is unclear.

### 3.1.7. *Alcohol withdrawal and cortisol responses to tasks*

Two trials involving alcohol did not specifically investigate the effects of alcohol drinking or abstinence on long-term cortisol levels; instead, they both examined alterations in the HPA axis in alcoholic patients in experimental conditions, after long-term alcohol withdrawal. Although the study designs were different, both trials found some evidence that serum cortisol responses to tasks were blunted in alcoholic participants compared with non-alcoholic ones (Coiro et al., 2007; Sinha et al., 2011).

### 3.1.8. *Caffeine*

Evidence is insufficient to determine the influence of caffeine intake on long-term levels of cortisol. No observational study has reported an association between caffeine consumption and long-term levels of cortisol. Only one interventional study examined cortisol responses measured in saliva to a caffeine challenge after controlled levels of daily caffeine intake over 4 weeks (Lovallo et al., 2005). They observed that taking caffeine capsules on the test day after 5 days of abstinence resulted in a significant elevation of cortisol throughout the entire day. Significant elevations of cortisol for about 6 hours on the test day were seen in the group that had caffeine intake of 300 mg for five days, but not 600 mg. Lovallo et al. (2005) concluded that daily caffeine intake caused a partial but not complete tolerance to caffeine's effects on cortisol secretion and that elevated cortisol levels may occur in the afternoon hours in those consuming repeated doses throughout the day.

## 3.2. *Mood correlates of cortisol*

Twenty-three observational studies reported a relationship between chronic levels of cortisol and mood factors including stress, anxiety, depression, psychological distress, post-traumatic stress disorder (PTSD), coping efficacy, psychological functioning, mindfulness, and fatigue.

### 3.2.1. *Perceived stress*

A total of 10 observational studies investigated the association between cortisol and self-reported perceived stress, with 10 using the Perceived Stress Scale (PSS) and one also using a computer task (Implicit Association Test). Four of the ten studies measured HCC over 3 months and self-reported perceived stress over the past month, among which three did not find a significant association, and one reported a weak negative correlation. One study measured HCC over the past 6 months and perceived stress over the past 2 months and did not find significant correlation. One study measured HCC and perceived stress over the same 3 months and did not find significant correlation. One study measured both HCC and perceived stress over the same month where HCC was reported to have no correlation with self-reported stress but was significant positively correlated with implicit stress measured by computer tasks. Two other studies measured HCC for longer periods of time in pregnant women. Hoffman et al. (2016) measured perceived stress with PSS at 16, 22, 28, 34, 40 weeks of gestation and HCC at 16, 28, 40 weeks of gestation. The researchers found that higher levels of first-trimester HCC were prospectively correlated with higher levels of perceived stress at 40 weeks of gestation and that the second-trimester HCC was positively correlated with perceived stress at 16 weeks of gestation. Another study took the maternal hair samples to measure HCC over the past 9 months and asked participants to complete the PSS during a clinical visit at 24–26 weeks of gestation. They did not find any association between HCC and perceived stress (Kramer et al., 2009). The final study measured long-term levels of cortisol over 1 month in saliva and self-reported perceived stress over the same month (Byun, 2013). They found that salivary cortisol on waking was not correlated with perceived stress, but salivary cortisol in the evening had a positive correlation with higher levels of perceived stress. Despite the mixed observations from these studies, a meta-analysis, which included data from eight studies Faresjö et al. (2013), Gerber et al. (2013), O'Brien et al. (2013), X. Chen et al. (2015), Sumra and Schillaci (2015), Geng et al. (2016), and Heinze et al. (2016), (Hoffman et al., 2016) showed a pooled correlation between long-term levels of cortisol and perceived stress ( $ESr = 0.114$ ,  $p = 0.0015$ ,  $CI = [0.023, 0.203]$ , Figure 4).

### 3.2.2. *Mood impact of stressors*

A total of three observational studies measured the association between cortisol and mood impact of stressors. The assessed stressors included negative life events in the previous up to 90 days, chaos in the neighbourhood in the past year, chaos/order of the home environment without a specified time frame, and mental burdening events in the past 3 months. All three studies measured HCC over 3 months. (Grassi-Oliveira et al., 2012; O'Brien et al., 2013; Ullmann et al., 2017) Two of the three studies reported that HCC was positively correlated with self-reported stressful life events or mental burdening events. (Grassi-Oliveira et al., 2012; Ullmann et al., 2017) One study did not find significant associations between HCC and chaos either in the neighbours or in the home. (O'Brien et al., 2013)

A meta-analysis based on available data from two studies (Grassi-Oliveira et al., 2012; O'Brien et al., 2013) showed that the association between stressors and HCC was not significant ( $ESr = 0.063$ ,  $p = 0.288$ ,  $CI = [-0.053, 0.177]$ , Figure 5). To summarise, two out of three studies found a significant positive association between HCC and stressors. However, this observation is not supported by the pooled results from the meta-analysis.

### 3.2.3. *Other indices of stress*

Three observational studies examined the associations between cortisol and other self-reported indices of stress. One study measured HCC over the 3 months before delivery in pregnant women and did not find a significant association between HCC and self-reported chronic stress over the same 3 months (Braig et al., 2016). One study measured salivary cortisol in pregnant women at 14, 20, 32 weeks of gestation and measured self-reported distress over the same period. They found that cortisol levels in saliva were positively correlated with distress among pregnant women (Giesbrecht et al., 2013). Finally, a study measured HCC and self-reported work stress over the past month and reported that neither the effort nor the reward scores were correlated with HCC

(Qi et al., 2014). However, they did find that the effort-reward imbalance ( $1.83 \times \text{effort-score} / \text{reward-score}$ ) at work was positively correlated with HCC. To summarise, two of the three studies reported a significant positive association between HCC and specific features of stress, namely distress and effort-reward imbalance at work. This observation was not, however, supported by the meta-analysis of three studies with a non-significantly pooled correlation between specific features of stress and HCC ( $ESr = 0.251$ ,  $p = 0.097$ ,  $CI = [-0.046, 0.508]$ , Figure 6).

#### 3.2.4. Anxiety

Five observational studies examined the association between cortisol and self-reported anxiety, with mixed findings. All of the studies measured cortisol using hair samples of 3 cm, despite different courses of time overall and different measures of anxiety. Three studies measured HCC over 3 months and none of them found a significant association between HCC either pregnancy-related anxiety over 3 months (Braig et al., 2016); not specified time frame (Kramer et al., 2009) or anxiety in general (not specified time frame, (Faresjö et al., 2013)). One other study measured both anxiety and HCC at 16, 22, 28, 34, 40 weeks of gestation and found that self-reported state-trait anxiety measured at 16 and 28 weeks of pregnancy was correlated with HCC in both the second and the third trimester (Hoffman et al., 2016). One study measured the HCC over 3–6 months and anxiety, which was measured for approximately 3 months within the aforementioned 3–6 months. This study did not find a significant association between HCC and anxiety (Heinze et al., 2016). A meta-analysis based on available data from three studies showed that the pooled correlation between long-term levels of cortisol and anxiety was not significant ( $ESr = 0.082$ ,  $p = 0.137$ ,  $CI = [-0.026, 0.187]$ , Figure 7).

#### 3.2.5. Depression

Twelve observational studies examined the association between long-term levels of cortisol and depression measured using seven different scales, half of which found a significant association between depression and cortisol. The ten studies measured HCC, among which three measured HCC using hair samples of 3 cm to 9 cm from pregnant women and did not find a significant association between HCC and depression, and one measured HCC over 6 months using two separate 3 cm samples and depression over the same 6 months, and did not find a significant association between HCC and depression. Two other studies measured HCC from the 3 months prior to conception to the third trimester, with one measuring depression over 1 week at each trimester (Hoffman et al., 2016) and one measuring depression shortly after delivery Caparros-Gonzalez et al. (2017) found that HCC during 1<sup>st</sup> and 3<sup>rd</sup> trimester was prospectively positively associated with depression. Hoffman et al. (2016) found that HCC during the 1<sup>st</sup> trimester was prospectively positively associated with depression at 40 weeks of gestation, and both 2<sup>nd</sup>- and 3<sup>rd</sup>-trimester HCC were positively associated with depression at 16 and 28 weeks of gestation. Four studies measured HCC over 3 months and depression over an unclear period, among which one reported a significant negative correlation between HCC and depression (Gerber et al., 2013), one reported a significant positive correlation between HCC and depression (Faresjö et al., 2013), and two reporting no association between HCC and depression (Schalinski et al., 2015; Wang et al., 2016). Two studies examined the association between cortisol measured in saliva over 1 month and depression, one of which found that cortisol in saliva was higher and the daily cortisol slope steeper in the depressed participants compared to the non-depressed ones (Booij et al., 2015). Another study found that although not significantly correlated with depression at the beginning of the study, elevated cortisol levels in saliva in the evening were positively correlated with self-reported depressive symptoms 4 weeks later (Byun, 2013).

Meta-analysis was performed with available data from seven studies, with a pooled correlation coefficient of 0.133 ( $p = 0.022$ ,  $CI = [0.019, 0.243]$ , Figure 8) suggests that long-term levels of cortisol, either measured in hair or saliva, were related to depression.

### 3.2.6. PTSD

Three observational studies examined the relationship between long-term levels of cortisol and PTSD, where cortisol and self-reported PTSD were measured differently. Cieslak et al. (2011) found that the PTSD at 1-month post-trauma was negatively associated with area under the curve with respect to increase (AUC<sub>i</sub>) at 3 months and that PTSD at 3 months was positively associated with area under the curve with respect to ground (AUC<sub>g</sub>) at 1-month post-accident. Stoppelbein and Greening (2015) measured salivary cortisol and self-reported PTSD over the same 12 months. They found a positive correlation between higher levels of cortisol and one of the self-reported PTSD symptoms: numbing symptoms. The third study measured HCC using 3 cm hair samples and clinically assessed the severity of PTSD symptoms over an unclear period and did not find a significant association between the two variables (Schalinski et al., 2015). Data were insufficient to conduct a meta-analysis and therefore it was not possible to interrogate the observation further.

## 3.3. Effects of mood interventions on cortisol

### 3.3.1. Mindfulness

Six mindfulness interventions were included in this review. All six studies measured long-term cortisol in saliva, and one also measured cortisol in blood. Three studies hypothesised reduced cortisol after intervention. K. W. Brown et al. (2016) reported that the mindfulness intervention significantly improved participants' perceived stress levels and mood, which did not significantly differ from the social support group during 3-month follow-up. Neither intervention showed changes in the diurnal cortisol response curve. Bergen-Cico et al. (2014) reported that participants who completed all sessions had a significant decrease in the cortisol awakening response (CAR) and AUC<sub>i</sub>, compared with the control group. They also reported significant associations between changes in cortisol levels and engagement in mindfulness intervention. Changes in mood were not described. Daubenmier et al. (2011) found significant improvement in mindfulness, anxiety, and external-based eating, but not in emotional eating or the average CAR in the mindfulness-based eating intervention group compared to that control participants. Neither group showed significant changes in the cortisol slope or morning serum cortisol concentrations. However, they found that obese participants in the intervention group showed significant reductions in the CAR and maintained body weight, which were not observed for obese participants in the control group. They also reported associations between reduced abdominal fat and improved self-reported mindfulness and chronic stress, and CAR in the intervention group. The three studies did not describe the hypothesised intervention effects on cortisol. Jacobs et al. (2013) found that self-reported mindfulness significantly increased after the intervention, but cortisol levels did not significantly change. However, they found that changes in mindfulness were inversely associated with changes in the average values of the afternoon and bedtime cortisol levels. Lynch et al. (2011) found significantly improved perceived stress, anxiety and depression among participants, along with increased self-reported mindfulness but no significant changes in cortisol levels after the intervention. Marcus et al. (2003) found a non-significant decrease in self-reported perceived stress after intervention among participants. However, the awakening salivary cortisol levels were significantly lower following the intervention. Half of the mindfulness interventions resulted in reductions in cortisol levels following the interventions, but half of the interventions did not find a significant treatment effect on cortisol. Therefore, whether mindfulness-based stress interventions can reduce cortisol levels remains unclear.

### 3.3.2. Social and emotional support

Holt-Lunstad et al. (2008) examined whether a support enhancement intervention could decrease salivary cortisol as an indicator of improved mood, with a behaviour monitoring control group where participants were instructed to just keep a diary of their physical affection and mood. Stress, mood, or social support were not measured. They did not find intervention effects on salivary cortisol.

### 3.3.3. *Other stress management interventions*

Three studies investigated the effects of other stress management methods on long-term levels of cortisol measured in saliva. Two studies did not describe the hypothesised direction of changes in cortisol. They both reported reduced stress (Limm et al., 2011; Tsiouli et al., 2014) and improved lifestyle (Tsiouli et al., 2014) or mood (Limm et al., 2011) but neither study observed significant changes in cortisol levels. They also found significant improvement in lifestyle factors, such as sleep, stress-related symptoms, and eating habits in both groups. One study hypothesised that participants in the stress-management intervention group would demonstrate lower intraindividual cortisol variability (i.e. the degree to which an individual's cortisol output may be erratic on a given day) measured in (Sannes et al., 2015). They only measured cortisol as the outcome in this study and reported lower levels of intraindividual cortisol variability among intervention participants compared to the waitlist control group. They also observed improvements towards a steeper cortisol slope and lower AUC<sub>G</sub> in the intervention group. However, they did not find significant changes in awakening cortisol levels, evening cortisol levels, and CAR following the intervention. At present the evidence for the effects of these stress-management interventions on cortisol is weak.

### 3.3.4. *Alternative therapies*

The two studies examined the effects of 5-week alternative therapies including acupuncture (Huang et al., 2012) and massage (Leivadi et al., 1999) on reducing stress and changing cortisol levels measured in saliva. Neither study showed significant intervention effects on long-term cortisol levels. The acupuncture intervention did not hypothesise the direction of changes in cortisol levels. Neither the decrease in perceived stress nor the increased CAR found in both the intervention and wait-list control groups were significant. No significant changes in the decline in cortisol or the daily average cortisol concentrations were found between groups following the intervention. The massage intervention hypothesised reduce cortisol levels and other potential stressors, such as anxiety and neck, shoulder, and back pain following the intervention. Participants reported reduced neck, shoulder, and back pain after the treatment sessions but changes in mood, anxiety, or cortisol levels were not observed over time although they found decreased cortisol levels immediately after the intervention sessions in both groups.

### 3.4. *Risk of bias*

For the current review, the maximum score of the checklist was 20 for observational studies and 27 for interventional studies, with four sub-sections: quality of reporting (maximum = 8 for observational studies, maximum = 10 for interventions), external validity (maximum = 3 for all of the studies), internal validity bias (maximum = 4 for observational studies, maximum = 7 for interventions), and confounding and selection bias (maximum = 4 for observational studies, maximum = 6 for interventions). The average total score was 9 out of 20 (range: 5–13) for observational studies and 11 out of 27 (range: 7–17) for interventions. Observational studies scoring 10 (n = 11) or above and interventions scoring 14 or above (n = 7) were considered to have moderate quality. The overall quality was considered moderate for observational studies and poor for interventional studies. A summary of the results for risk of bias assessment is presented in Supplementary Appendix Table 3.

## 4. Discussion

The main aim of this review was to systematically identify and review the available literature on the associations between lifestyle and mood factors and long-term levels of cortisol in healthy adults. The quality and scientific rigour of research conducted over the last two decades in this area were assessed. Overall, the evidence for associations between lifestyle and long-term levels of cortisol was mixed, with considerable heterogeneity between studies on approaches to assessing cortisol, lifestyle and mood factors, and with observational studies meeting criteria for moderate quality and intervention studies meeting criteria for poor quality.

Nonetheless, we can, in this review, glean some evidence in support of a positive association between physical activity and long-term levels of cortisol, with the meta-analysis of observational

studies showing a significant pooled correlation. Although the evidence from trials also seemed to suggest that physical activity is likely to induce changes in cortisol levels over time, the direction of the changes remained unclear due to mixed findings. The meta-analysis for observational studies also showed a significant pooled positive correlation between BMI/body weight and long-term levels of cortisol. However, the only trial, which aimed to reduce weight through diet with and without exercise, did not find changes in cortisol levels. Other dietary interventions, regardless of whether they induced changes in body weight or body composition or not, were unlikely to induce changes in long-term cortisol levels. Observational studies suggested that perceived stress and depression were likely to be positively associated with long-term levels of cortisol. However, the observational evidence does not support a significant association between stressors, specific features of stress and anxiety, and cortisol. PTSD is likely to be positively associated with cortisol, although this conclusion is tentative due to insufficient data. Overall, trial evidence examining the effects of psychological interventions on long-term levels of cortisol was insufficient and mixed. Finally, evidence from both observational and interventional studies was insufficient to draw a clear conclusion for the associations between long-term levels of cortisol and smoking, sleep, alcohol consumption, and caffeine consumption.

#### **4.1. Heterogeneity in how lifestyle and mood were measured**

One observation from this review was the variability in how lifestyle and mood were measured across studies, which may have contributed to the mixed findings. For example, all three of the observational studies examining the association between physical activity and HCC measured physical activity differently. Due to different types of lifestyle measures being used in these studies, it is difficult to draw a definitive conclusion whether these lifestyles are correlated with cortisol, regardless of findings reported by the studies. Similar to the lifestyle measures, specific mood measures assess different aspects of mood. For example, the PSS measures the general distress and inability to cope (Cohen et al., 1983) whereas the Effort-Reward Imbalance Scale measures work stress with a focus on the efforts invested into job performance and rewards received in turn (Siegrist, 1996). The associations between cortisol and various mood outcomes might not be consistent (e.g. the pooled correlation is significant for cortisol and perceived stress and depression but not for cortisol and anxiety), indicating that the nature of the mood under investigation, and the source of the negative mood experienced, may be important.

#### **4.2. Heterogeneity in approaches to measure cortisol**

Another reason for the mixed findings might be the considerable variety of how long-term levels of cortisol were measured across studies: the type of cortisol measured, the indices examined, and the sampling protocol adopted.

First, different types of cortisol were measured both overall and in studies examining the same lifestyle or mood factor. For example, 80% of the observational studies measured cortisol in hair, other 20% measured cortisol in saliva whilst out of the 27 trials, only 1 measured cortisol in hair, 1 in urine, 6 in blood, and 19 in saliva. Cortisol measured in different samples reveals different information. Although salivary cortisol is often used as an alternative to free cortisol measured in blood, evidence suggested that the correlation between salivary and free serum cortisol varies between different individuals for daily-paired samples (Levine et al., 2007). Salivary and urinary cortisol measured over the same 24 h window were likely to provide different information about cortisol secretion (Yehuda et al., 2003), and how comparable is hair cortisol to saliva/urine/blood cortisol is still under debate (Kalliokoski et al., 2019). Results from this review echoes evidence that cortisol measured in different samples is hardly comparable and should not be used interchangeably (Hellhammer et al., 2009; Van Holland et al., 2012; Yehuda et al., 2003). Secondly, a variety of the cortisol indices were measured, even when studies did measure the same type of cortisol. For example, although all of the six mindfulness interventions measured cortisol in saliva, two measured awakening salivary cortisol and both reported significant intervention effects on cortisol (Bergen-Cico et al., 2014; Marcus et al., 2003), whereas others where significant intervention effects on cortisol were not found measured diurnal cortisol (K. W. Brown et al., 2016), resting p.



m. cortisol values (Jacobs et al., 2013), or did not specify the measured cortisol indices (Daubenmier et al., 2011; Lynch et al., 2011). Different cortisol indices capture different characteristics of cortisol activity and should not be used interchangeably (Vedhara et al., 2006). With a limited number of studies measuring the effect of the same lifestyle/mood intervention on the same index of cortisol, it is therefore difficult to conclude whether a certain type of intervention affects cortisol or whether the effect, if any, will only be revealed when measured by certain indices of cortisol. This issue has pointed out an important field to work on urgently: to identify which cortisol indices should be focused on while examining the influence of lifestyle and mood. Thirdly, studies adopted a variety of sampling procedures. For example, although four 8-week mindfulness interventions all measured cortisol in saliva, the time samples were collected, number of samples collected per day, and for how many consecutive days, differed. The accuracy and stability of cortisol outcomes depend highly on the sampling protocol when sampled in acute measures such as blood and saliva, which includes the exact timepoint of sampling, the frequency, the length of the sample collection period, and the adherence (Daubenmier et al., 2011; Saxbe, 2008; Turpeinen & Hämäläinen, 2013). Different sampling protocols could bring significant variations of the cortisol results to different studies (Clow et al., 2004). Furthermore, lack of clarity about the rigour in timing and sufficiency of sampling in studies failed to report such details could both contribute to the inconsistency in the evidence. These observations further indicated that the ability of these acute measures to reflect the chronic relationship between lifestyles/mood and cortisol or the enduring treatment effects on cortisol is limited. The sampling procedure for hair cortisol is relatively straightforward and consistent across studies: hair samples of a certain length were taken close to the scalp from the posterior vertex area of the head. In this review, we did find significant pooled correlations between HCC and some of the lifestyle and mood factors such as BMI and perceived stress. This suggests that HCC might be a promising method to examine the long-term relationship between cortisol and lifestyle and mood.

#### **4.3. The temporal association between cortisol and lifestyle or mood**

It was observed in this review that although several studies measured cortisol and lifestyle/mood concurrently, the time frames captured by the measures did not necessarily correspond. For example, three observational studies examined physical activity and HCC all measured HCC over the previous 3 months, but two of the studies measured physical activity over the past week (Gerber et al., 2013; Sumra & Schillaci, 2015) and one asked participants whether or not they had engaged in physical activity regularly without specifying a time-frame (Garcia-Leon et al., 2018). This brought the issue that, in these studies, whether the examined associations of cortisol and lifestyle/mood reflected the actual association over the time frame when cortisol was captured, or when lifestyle/mood was measured was unclear. Therefore, future studies should clearly report the time frames these variables were measured and cautiously interpret the results. Two studies included in this review found an association between cortisol and subsequent mood, both reporting higher HCC prediction of later-measured depression (Caparros-Gonzalez et al., 2017; Hoffman et al., 2016). Similar associations between cortisol and major depressive disorder have been reported previously. For example, higher baseline CAR was identified as a significant risk factor for major depressive disorder in adolescents (Adam et al., 2010; Ellenbogen et al., 2011; Vrshek-Schallhorn et al., 2013). There is also evidence suggesting that individual differences during the morning salivary cortisol levels may be a risk factor for subsequent major depressive disorder among women (Harris et al., 2000). One study in this review reported an association between mood and subsequent cortisol: higher levels of perceived stress, anxiety, and depression at 16 weeks of gestation were significantly correlated with higher levels of HCC over the second trimester (Hoffman et al., 2016). These findings suggested that our understanding of the relationship between cortisol and lifestyle and mood would benefit from prospective studies conducted over long time frames in which it is possible to explore not only the concurrent relationship between these factors, but also conduct time-series analyses to help to disentangle cause and effect, or more likely the circular relationship between cortisol, lifestyle, and mood.

#### **4.4. Other issues from the interventions**

The overall picture of the effects of lifestyle or psychological interventions on long-term levels of cortisol was mixed, with only 18 out of 27 trials showing significant effects on long-term levels of cortisol. The insufficient or mixed evidence and methodological limitations. First, most of the trials described cortisol as a biomarker of mood hypothesising that the interventions would influence mood, which might be captured by cortisol. However, this hypothesised mechanism was not always affected in these studies, for instance, one stress-reduction intervention where lower awakening salivary cortisol levels were found did not result in significant improvement in self-reported stress (Marcus et al., 2003). A few studies did not measure or fully report changes in the targeted mechanisms. For example, one of the two yoga studies only measured stress using cortisol but not self-reported stress (P. J. Chen et al., 2017). Similarly, one intervention targeted at stress among veterans with PTSD did not report changes in stress or PTSD (Bergen-Cico et al., 2014). Cortisol levels, captured either short term or long term, may not correlate well with self-reported mood measures, hence these different measures should not be treated interchangeably (Van Holland et al., 2012). If an intervention did not measure, report or result in any changes in the hypothesised mechanism, regardless of whether the effect on cortisol was significant, it is impossible to distinguish whether it was indeed due to the intervention being effective/ineffective or other reasons. Second, many trials failed to hypothesise the direction of intervention effects on cortisol. Out of the 27 trials, less than half ( $n = 12$ ) hypothesised the direction of the effect on cortisol, only half of these ( $n = 6$ ) reported findings that were in line with their hypotheses (Bergen-Cico et al., 2014; Daubenmier et al., 2011; Goldberg et al., 2014; Miller et al., 2015); reductions during the evening cortisol (Papacosta et al., 2013; Sannes et al., 2015). The other six studies, which did hypothesise a direction either did not find a significant intervention effect on cortisol (Diment et al., 2012; Holt-Lunstad et al., 2008; P. J. Chen et al., 2017; Leivadi et al., 1999; K. W. Brown et al., 2016) or found it in the opposite direction (Tam et al., 2014). Given that there are a limited number of studies reporting significant effects in the expected direction, it is difficult to conclude whether a specific type of intervention is effective in changing long-term cortisol levels. However, the six interventions that confirmed their hypotheses had clearer descriptions of which cortisol indices they measured. It is likely that a lifestyle or mood intervention that changes cortisol levels may benefit from a more specific hypothesis involving the indices of cortisol and the direction of possible effects.

#### **4.5. Strengths and limitations**

This is the first systematic review to synthesise the available literature on the associations between lifestyle and mood factors and long-term cortisol levels in healthy adults. This is also the first systematic review to synthesis relevant evidence from both observational and interventional studies. By doing so, it contributes to the understanding of the role of cortisol, when assessed over the long term, in lifestyle and mood. This review also points to the outstanding gaps between and within observational and interventional studies in cortisol research, which have revealed several directions for future research. Another strength of this review is the robust methods that were used throughout the review process, and the quality as well as the scientific rigour of studies were assessed taking into consideration both the study design and reporting. However, the studies included in this review were highly heterogeneous, thus the meta-analytic approach was only adopted for observational studies of BMI, physical activity, perceived stress, stressors, and other indices of stress, anxiety, and depression. Findings from the meta-analyses led to the conclusion that BMI, physical activity, perceived stress, and depression were significant correlates of chronic levels of cortisol. However, due to the mixed findings in relevant trials, this conclusion should be interpreted with caution. Another limitation should be noted that the overall quality of reviewed studies was only moderate for observational studies and poor for interventional studies. The lack of quality was salient in the external validity and bias in both the design and reporting of the studies, which further emphasised the need for robust research in this area. As was pointed out by a previous review, the lack of consensus within the literature regarding what cortisol outcomes are most meaningful to health is still a challenge in cortisol research (Saxbe, 2008). An urgent step for future research will be to address the limitations and concerns discussed in this review and to



rethink our knowledge of cortisol being a biomarker of stress and behaviour. We were unable to duplicate the title and abstract screening due to the size of the search. However, a second reviewer was involved in full-text screening, which increased the certainty that relevant papers were included.

#### 4.6. Recommendations for future research

Finally, several recommendations for future research are made. First, more research is needed to investigate the relationships between long-term cortisol levels and sleep, smoking, alcohol consumption, and caffeine consumption. Nonetheless, there is emerging evidence for a positive association between BMI/body weight, physical activity, perceived stress and depression and cortisol, suggesting that intervention trials in these areas may be fruitful areas of enquiry. Such trials would benefit from (1) using more consistent protocols to measure cortisol over the longer terms, such as HCC; (2) clearer description of the hypotheses and aims of the study in relation to cortisol, (3) measurement of cortisol levels at baseline or over longer periods pre-intervention as reference. Hypothesised mechanisms (e.g. the mediating mood factors) for the interventions to be effective should also be clearly described, measured, and reported. For the association between cortisol and mood, it is possible that the long-term association between these two factors is prospective. Current evidence to support this observation, however, is insufficient. Future research is encouraged to explore this area with two focuses: (1) the prospective association itself and (2) whether it is mood that predicts cortisol or vice versa.

#### Author details

Ru Jia<sup>1</sup>

E-mail: [ru.jia@nottingham.ac.uk](mailto:ru.jia@nottingham.ac.uk)

ORCID ID: <http://orcid.org/0000-0001-6843-8250>

Sophie Carlisle<sup>1</sup>

ORCID ID: <http://orcid.org/0000-0002-9784-9910>

Kavita Vedhara<sup>1</sup>

ORCID ID: <http://orcid.org/0000-0002-9940-7534>

<sup>1</sup> School of Medicine, University of Nottingham, University Park, Nottingham, NG7 2RD, UK.

#### Disclosure statement

KV is a member of the independent scientific advisory board for Cortigenix.

#### Author contributions

All authors contributed to the study's design. RJ and SC contributed to the systematic searches and data extraction processes. RJ drafted the first draft of the manuscript. All authors contributed to and approved the final version of the manuscript.

#### Data Availability Statement

All available data including the data extraction tables for all included studies, and raw data for meta-analysis are available here: <https://osf.io/36fnt/>

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