Short- and Long-Term Effects of Acute Stress and HPA-Axis (Re)activity: New Findings for Healthy Individuals, Hypertensives, and Patients with Coronary Heart Disease

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To my parents

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Record of Achievement and Individual Contributions

Published articles of this thesis and individual contributions are listed in the following.

Publications

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Summary

Acute psychosocial stress has a variety of short-term physiological, cognitive, and behavioral effects. In the long term, these effects can pose an increased risk to well-being and health. In particular, stress-related physiological changes are associated with a heightened cardiovascular health risk, such as the development of coronary heart disease (CHD). Vulnerable groups, including hypertensives or CHD-patients, could be particularly affected by the consequences of stress and associated physiological changes. However, the underlying mechanisms of the short- and long-term effects of stress at different levels are not yet fully understood. The hypothalamic-pituitary-adrenal (HPA-) axis and its end-product cortisol are central elements of the acute stress response and overall homeostasis that may mediate the effects of stress and possibly contribute to adverse health outcomes. The overall aim of this thesis was to investigate the biological mechanisms of different short- and long-term effects of acute psychosocial stress and HPA-axis (re)activity in different groups of individuals. To this end, two cross-sectional and two longitudinal studies were conducted.

The first study focused on the short-term effects of acute stress and related changes in cortisol levels on the concentration performance of healthy men. The study participants in the experimental (stress) group improved more in their concentration performance after stress induction compared to a control group that had not been exposed to acute stress. This improvement was associated with lower increases in cortisol levels. The second study investigated endocrine mechanisms of eating behavior after acute stress in healthy individuals, as well as possible sex-specific differences. The results indicated an association between lower cortisol stress reactivity and a higher feeling of hunger in both men and women. The study also suggested that women may be at a higher risk of stress-induced eating, as they had lower overall cortisol levels and no reactivity (neither to stress nor to eating) in the satiety hormone cholecystokinin. A further focus of the thesis included the investigation of the long-term effects

of acute stress and the basal HPA-axis activity on the cardiovascular health of healthy individuals, hypertensives, and CHD-patients. The third study addressed the clinical significance of stress reactivity of blood lipids in male healthy normotensives and essential hypertensives. The analyses aimed to identify possible group differences in (hemoconcentration-corrected) blood lipid stress reactivity. Prospective associations of blood lipid stress reactivity with intermediate biological CHD risk factors were also investigated. The results indicated a higher blood lipid reactivity to acute psychosocial stress in hypertensives as compared to normotensives. Furthermore, the blood lipid stress reactivity predicted increases in CHD risk after an average of 3 years. The fourth study examined the potential clinical implications of basal HPA-axis activity in male healthy normotensives, essential hypertensives, and CHD-patients. Assumed group differences in the basal (diurnal) cortisol secretion were examined before longitudinal associations with intermediate biological CHD risk factors were investigated. The results pointed to a down-regulation of the basal HPA-axis activity in CHD-patients and hypertensives compared to normotensives. Furthermore, a lower basal HPA-axis activity was found to be a predictor of increased CHD risk after approximately 3 years.

Taken together, the findings of the present thesis highlight the importance of addressing the short- and long-term effects of stress and the underlying biological mechanisms in basic and clinical research and provide a basis for practical applications.

Zusammenfassung

Akuter psychosozialer Stress hat vielfältige kurzfristige physiologische, kognitive und verhaltensbezogene Auswirkungen. Diese können langfristig ein erhöhtes Risiko für das Wohlbefinden und die Gesundheit darstellen. So werden insbesondere durch Stress bedingte physiologische Veränderungen mit einem erhöhten kardiovaskulären Gesundheitsrisiko wie dem Auftreten der koronaren Herzkrankheit (KHK) in Verbindung gebracht. Vulnerable Gruppen wie etwa Personen mit Hypertonie oder KHK-Patienten, könnten somit besonders von den Effekten von Stress und den damit assoziierten physiologischen Veränderungen betroffen sein. Die zugrunde liegenden Mechanismen der kurz- und langfristigen Auswirkungen von Stress auf unterschiedlichen Ebenen sind jedoch noch nicht vollständig geklärt. Die Hypothalamus-Hypophysen-Nebennierenrinden (HHN-) Achse und ihr Endprodukt Cortisol sind zentrale Elemente der akuten Stressreaktion und der allgemeinen Homöostase, welche die Effekte von Stress vermitteln und sich nachteilig auf die Gesundheit auswirken könnten. Das übergeordnete Ziel dieser Arbeit war es, die biologischen Mechanismen unterschiedlicher kurz- und langfristiger Effekte von akutem psychosozialem Stress und der HHN-Achsen(re)aktivität in verschiedenen Personengruppen zu untersuchen. Zu diesem Zweck wurden jeweils zwei Quer- und zwei Längsschnittstudien durchgeführt.

In der ersten Studie standen die kurzfristigen Auswirkungen von akutem Stress und den damit einhergehenden Veränderungen der Cortisolspiegel auf die Konzentrationsleistung von gesunden Männern im Mittelpunkt. Die Teilnehmer der Experimental- (Stress-) Gruppe verbesserten ihre Konzentrationsleistung stärker nach der Stressinduktion als eine Kontrollgruppe, die keinem akuten Stress ausgesetzt gewesen war. Diese Verbesserung war mit einem geringeren Anstieg der Cortisolspiegel assoziiert. Die zweite Studie untersuchte endokrine Mechanismen des Essverhaltens nach akutem Stress bei gesunden Personen sowie mögliche geschlechtsspezifische Unterschiede. Die Ergebnisse deckten einen Zusammenhang

zwischen einer geringeren Cortisol-Stressreaktivität und einem höheren Hungergefühl bei Männern und Frauen auf. Da Frauen insgesamt niedrigere Cortisolspiegel und keine Reaktivität (weder auf Stress noch auf Essen) des Sättigungshormons Cholecystokinin aufwiesen, deutete die Studie zudem auf ein höheres Risiko für stressbedingtes Essen bei Frauen hin. Ein weiterer Schwerpunkt der Arbeit beinhaltete die Untersuchung der langfristigen Auswirkungen von akutem Stress und der basalen Aktivität der HHN-Achse auf die kardiovaskuläre Gesundheit von gesunden Personen, Hypertonikern sowie KHK-Patienten. In der dritten Studie stand daher die klinische Bedeutsamkeit der Stressreaktivität von Blutlipiden bei männlichen, gesunden Normotonikern und essenziellen Hypertonikern im Mittelpunkt. Die Analysen zielten darauf ab, mögliche Gruppenunterschiede in der (für Hämokonzentration korrigierten) Blutlipid-Stressreaktion zu ermitteln. Anschließend wurden prospektive Zusammenhänge der Blutlipid-Stressreaktivität mit intermediär-biologischen KHK-Risikofaktoren geprüft. Die Ergebnisse wiesen auf eine höhere Blutlipid-Reaktivität nach akutem psychosozialem Stress bei Hypertoniker im Vergleich zu Normotonikern hin. Zudem sagte die Blutlipid-Stressreaktion einen Anstieg des KHK-Risikos nach durchschnittlich 3 Jahren voraus. Die vierte Studie untersuchte die potenziellen klinischen Auswirkungen der basalen HHN-Achsenaktivität bei männlichen, gesunden Normotonikern, essenziellen Hypertonikern und KHK-Patienten. Zunächst wurden vermutete Gruppenunterschiede in der basalen (diurnalen) Cortisolsekretion untersucht, bevor längsschnittliche Zusammenhänge mit intermediär-biologischen KHK-Risikofaktoren geprüft wurden. Die Ergebnisse deuteten auf eine Herabregulierung der basalen HHN-Achsenaktivität bei KHK-Patienten und Hypertonikern im Vergleich zu Normotonikern hin. Darüber hinaus erwies sich eine niedrigere basale HHN-Achsenaktivität als Prädiktor für ein erhöhtes KHK-Risiko nach etwa 3 Jahren. Zusammenfassend unterstreichen die Ergebnisse der vorliegenden Arbeit die Bedeutung der Untersuchung kurz- und langfristiger Effekte von Stress und der zugrunde liegenden biologischen Mechanismen in der grundlagenbezogenen und klinischen Forschung und bieten eine Basis für praktische Anwendungen.

List of Abbreviations

A	ABI	ankle-brachial index
	ACTH	adrenocorticotropic hormone
	AN(C)OVA	analysis of (co)variance
	ARC	arcuate nucleus
	AUC_G	area under the curve with respect to the ground
	AVP	arginine vasopressin
B	BMI	body mass index
	BP	blood pressure
C	CABG	coronary artery bypass graft
	CAC	coronary artery calcification
	CAR	cortisol awakening response
	CCK	cholecystokinin
	CHD	coronary heart disease
	CNS	central nervous system
	CPT	Cold Pressor Test
	CRH	corticotropin-releasing hormone
	CRP	C-reactive protein
	CVD	cardiovascular diseases
D	DALYs	disability-adjusted life years
	DBP	diastolic blood pressure
E	EPI	epinephrine
G	GC	glucocorticoids
	GR	glucocorticoid receptors
H	HbA1c	glycosylated hemoglobin A1c
	HDL(-C)	high-density lipoprotein (cholesterol)
	HPA	hypothalamic-pituitary-adrenal
	HHN	Hypothalamus-Hypophysen-Nebennierenrinden
	HR	heart rate
	HT	hypertensives
I	IL-6	interleukin-6
K	KHK	koronare Herzkrankheit
L	LDL-C	low-density lipoprotein cholesterol

	LVEF	left ventricular ejection fraction
M	MAN(C)OVA	multiple analyses of (co)variance
	MAP	mean arterial blood pressure
	MI	myocardial infarction
	MIST	Montreal Imaging Stress Task
	MR	mineralocorticoid receptors
N	NE	norepinephrine
	NT	normotensives
	NTS	nucleus of tractus solitarius
P	PVN	paraventricular nucleus
S	sAA	salivary alpha-amylase
	SAM	sympathetic-adrenal-medullary
	SBP	systolic blood pressure
	sCPT	socially evaluated Cold Pressor Test
	SMCs	smooth muscle cells
	SNS	sympathetic nervous system
T	TC	total cholesterol
	tChol	total cholesterol
	TG	triglycerides
	TNF-α	tumor necrosis factor α
	TSST(-G)	Trier Social Stress Test (for groups)
V	VIF	variance inflation factor

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Introduction

Psychosocial stress is a very common condition in our everyday lives (e.g., Gallup, 2023; Zawadzki et al., 2019) that can significantly impact a person's well-being and health (Schneiderman et al., 2005). Specifically, stress involves the adaption to various challenging situations (McEwen, 2006) with a broad range of possible short- and long-term consequences for the individual (Chrousos, 1998; McEwen & Stellar, 1993). How stress affects us can vary considerably (e.g., Dantzer, 2016). It has been suggested that the way the mind and particularly the body respond to demanding situations determines the consequences of stress (Epel et al., 2018).

The short-term effects of acute stress are supposed to facilitate adaptive and to inhibit not-required functions on physiological, cognitive-affective, and behavioral levels (Chrousos & Gold, 1992; Epel et al., 2018; McEwen, 1998a). These changes are intended to be protective and to maintain performance, ultimately allowing the survival of the organism (Dhabhar, 2018; Romero & Butler, 2007). It has further been reported that acute stress can have negative effects in the short term that may affect health in the long term (McEwen, 1998a). Similarly, stress has been shown to impair several cognitive processes (Calvo & Gutiérrez-García, 2016; Sandi, 2013) and to promote coping strategies such as substance abuse or increased eating that may affect health indirectly (Epel et al., 2018; O'Connor et al., 2021). The adaptions to acute stress are orchestrated (among others) by the stress reactivity of the hypothalamic-pituitary-adrenal (HPA-) axis and its end-product, cortisol (McEwen & Seeman, 1999; Romero & Butler, 2007). Investigating the underlying mechanisms in terms of the HPA-axis reactivity may help to improve our understanding of the short-term stress effects.

Regarding the long-term effects of stress, a substantial body of evidence has linked (chronic) stress to mental and somatic disorders (e.g., Cohen et al., 2016; Guidi et al., 2021). Stress is a well-documented risk factor, particularly for cardiovascular diseases (CVD),

including coronary heart disease (CHD) and the underlying process of atherosclerosis (Black & Garbutt, 2002; Kivimäki & Steptoe, 2018; Lu et al., 2013; Rosengren et al., 2004; Steptoe & Kivimäki, 2012). In addition, stress has been repeatedly associated with hypertension (Liu et al., 2017; Sparrenberger et al., 2009; Spruill, 2010)—an important factor that increases CHD risk (e.g., Whelton et al., 2018). The global burden of disease resulting from both CHD and hypertension is significant (Mensah et al., 2023). Understanding the role of common psychosocial risk factors, such as stress, in the disease development and progression in healthy and vulnerable individuals is therefore crucial. In addition to the indirect pathways linking stress to disease risk (e.g., via changes in health behaviors), heightened reactivity to acute stress (e.g., Treiber et al., 2003) and alterations in the regulation of the basal HPA-axis activity (Matthews et al., 2006) may play a role. These alterations can lead to changes in related biological systems, thereby increasing the risk for several diseases, including CHD (Cohen et al., 2007; Juster et al., 2010; McEwen, 1998b; McEwen & Seeman, 1999). However, their clinical relevance for the pathogenesis of CHD and atherosclerosis in healthy normotensive and hypertensive individuals, as well as CHD-patients is still under investigation.

For this reason, basic and clinical researchers have recognized stress as an increasingly relevant target for investigation (Godoy et al., 2018) that needs further elucidation. A deeper understanding of the underlying biological mechanisms of short- and long-term effects of acute stress and HPA-axis (re)activity in different study populations could offer important theoretical and practical implications. The thesis aimed to elucidate the endocrine mechanisms, in terms of HPA-axis reactivity, underlying acute stress effects on a cognitive and behavioral level. As preclinical research is required to understand the stress effects and related mechanisms (Allen et al., 2014), the first two studies of the thesis investigated the short-term impact of acute psychosocial stress and HPA-axis reactivity on concentration in a cognitive task and eating behavior respectively in apparently healthy individuals. The thesis aimed further to gain new

insights into the biological mechanisms underlying the long-term effects of acute psychosocial stress and basal HPA-axis activity on cardiovascular health. Two additional studies therefore investigated the potential effects of acute stress and basal HPA-axis activity on intermediate biological risk factors for CHD. The studies included healthy individuals with normal blood pressure (BP) levels, hypertensives, as well as CHD-patients.

The following chapters of the introduction present an overview of the basic concepts of stress research, followed by a detailed discussion of concentration performance, eating behavior, and CHD (risk factors). Furthermore, the current state of scientific evidence regarding the consequences of stress and the possible relevance of HPA-axis (re)activity will be reviewed. The introduction is followed by a presentation of the four studies on the short-term and long-term effects of acute stress and HPA-axis (re)activity. The thesis concludes with a general discussion of the obtained findings and their implications for further research and practical application.

Definitions and Concepts of Stress

The term “stress” is commonly used to refer to increased physical or mental strain or demands in everyday language. In the scientific community, however, efforts to find a satisfactory definition of stress have failed (e.g., Del Giudice et al., 2018; Kagan, 2016; Murison, 2016), and scientific stress concepts have thus constantly been revised and developed. As a consequence, current definitions of stress have emerged from different traditions in stress research, notably from epidemiological, psychological, and biological concepts (Cohen et al., 2016).

The epidemiological approach to stress research is based on the assumption that a specific life event produces the same level of stress in all individuals (Cohen et al., 2016). According to this stimulus-oriented definition, stress corresponds to the internal or external

stimuli that trigger the stress process (Reif et al., 2021; Werner et al., 2012). The amount of stress is cumulative, being determined by the number of changes required to adapt to a stimulus (Cohen et al., 2016; Holmes & Rahe, 1967). Thus, every additional event contributes to an individual's overall burden (Cohen et al., 2016; Holmes & Rahe, 1967).

The observation that a specific event is perceived as stressful by some individuals but not by others is the core of the psychological tradition of stress research (Cohen et al., 2016). Stress can derive from a dynamic person-environment relationship, with the individual's cognitive appraisal of a potentially stressful situation playing a central role (Lazarus, 1966; Lazarus & Folkman, 1984). According to the transactional stress model, stress results from an imbalance between perceived situational demands and individual coping resources (Lazarus, 1966; Lazarus & Folkman, 1984).

The biological tradition focuses on the physiological reactions that support the short-term adaptation to stress but may lead to increased vulnerability to diseases in the future (Cohen et al., 2016; Goldstein & Kopin, 2007; Schulkin, 2011). Response-oriented concepts of stress derive from Claude Bernard's work in the 19th century on the ability of an organism to maintain a stable organic environment, the milieu intérieur (Bernard, 1957; Goldstein & Kopin, 2007). Building up on Bernard's work, Walter B. Cannon developed the concept of homeostasis to describe the property of physiological systems to preserve a dynamic equilibrium despite external influences (Cannon, 1929; Cannon, 1939; Godoy et al., 2018; Goldstein & Kopin, 2007). Cannon had observed specific physiological reactions in the face of stressful situations, allowing the body to prepare for the so-called fight-or-flight response (Cannon, 1915; Godoy et al., 2018; Goldstein & Kopin, 2007). Hans Selye introduced the word "stress" with its current meaning (Chrousos, 2009) to describe a non-specific reaction of the body in response to any kind of burden, what he referred to as the General Adaptation Syndrome (Godoy et al., 2018; Goldstein & Kopin, 2007; Selye, 1950; Selye, 1976). Sterling and Eyer (1988) elaborated the

concept of homeostasis and coined the term “allostasis” to describe the ability of an organism to adapt to perceived and anticipated demands, maintaining physiological stability through change. Allostatic load describes the accumulated effects of repeated or chronic stress that is accompanied by fluctuating or heightened biological alterations (McEwen, 1998b; McEwen & Stellar, 1993), leading “to chronic wear and tear” (McEwen & Stellar, 1993, p. 2094). McEwen (1998b) identified different types of stress response profiles leading to allostatic load: the “(1) frequent activation of allostatic systems; (2) failure to shut off allostatic activity after stress; (3) inadequate response of allostatic systems leading to elevated activity of other, normally counter-regulated allostatic systems after stress” (p. 33). Allostatic overload results from the culmination of the dysregulations of biological systems, leading to negative health outcomes (Guidi et al., 2021; Juster et al., 2010; McEwen & Wingfield, 2003).

Stress can thus be defined as a “deviation from some norm or steady state” (Lazarus, 1993, p. 4) that is induced by a threat to a person’s well-being (Kagan, 2016). These intrinsic or extrinsic challenges or stressors can be psychological or physical and vary in their magnitude and chronicity (Chrousos, 1998; Chrousos, 2009). Physiological adaptation to a stressor is no longer considered a general response of the body, but the extent of the stress response depends primarily on the individual’s appraisal of the stressor and his or her resources (Fink, 2016; Lazarus, 1966; Lazarus & Folkman, 1984; Mason, 1971). Stress can be classified as acute or chronic, with acute or short-term stress lasting minutes to hours while chronic stress is experienced over a sustained period of weeks or months (Calvo & Gutiérrez-García, 2016; Dhabhar, 2018). Accordingly, stressors can take the form of acute life events, long-term challenges, and daily hassles (Steptoe & Kivimäki, 2012). The inner balance after acute stress is re-established by the stress response, a set of short-term physiological adaptions with effects on different levels of the organism that is orchestrated by the stress system (Chrousos, 1998).

The Stress System and the Biological Stress Response

The stress system includes both central nervous system (CNS) and peripheral components (Charmandari et al., 2005). The brain structures responsible for detecting events and interpreting them as potential stressors trigger the physiological response to threats (Godoy et al., 2018). In detail, stressors are processed in areas of the limbic forebrain, such as the prefrontal cortex, the amygdala, and the hippocampus (Ulrich-Lai & Herman, 2009). These structures are interconnected with (sub)cortical areas involved in higher-order cognitive functions (e.g., memory) and with brainstem structures, like the locus coeruleus or the raphe nuclei, which are known to be engaged in attention and arousal (Ulrich-Lai & Herman, 2009).

The processing of physical and psychological stressors involves distinct (partly overlapping) neural pathways, with the paraventricular nucleus (PVN), which is located in the hypothalamus, playing a crucial role (Godoy et al., 2018; Herman et al., 2003; Ulrich-Lai & Herman, 2009). This neural integration of stressor-related information can elicit the activation of the two main arms of the stress response, the sympathetic-adrenal-medullary (SAM-) axis and the HPA-axis (Godoy et al., 2018). The activation of the SAM- and HPA-axis leads to the release of some of the primary stress mediators, i.e., catecholamines and cortisol, allowing short-term adaptations to challenges (Juster et al., 2010; McEwen, 2003).

Sympathetic-Adrenal-Medullary Axis and Catecholamines

The SAM-axis is the first and fastest arm of the stress system to be activated in response to an encounter with a stressor (O'Connor et al., 2021), providing rapid physiological adaptations and rendering the body in a state of alert (Godoy et al., 2018). The stimulation of the sympathetic nervous system (SNS) leads to the release of norepinephrine (NE) from sympathetic nerve endings and elicits chromaffin cells of the adrenal medulla to synthesize and release predominantly epinephrine (EPI) and a smaller proportion of NE (Godoy et al., 2018;

Tank & Lee Wong, 2015). Catecholamines (i.e., NE and EPI) are produced before stress and stored in secretory vesicles, facilitating their immediate release after the detection of a stressful stimulus (Romero & Butler, 2007). The catecholamines bind to membrane-bound G-protein-coupled receptors in their target tissues, thereby initiating fast cellular responses (Romero & Butler, 2007).

The adaptions mediated by the SAM-axis represent the classic fight-or-flight response leading to effects on the cardiovascular, metabolic, and immune systems (Brotman et al., 2007; Romero & Butler, 2007; Ulrich-Lai & Herman, 2009). These adaptions include arousal and increases in BP, cardiac output, heart rate (HR), and respiration, as well as an enhanced energy supply (Tank & Lee Wong, 2015).

The activity of the SAM-axis can be assessed by the repeated measures of EPI and NE from plasma samples or by the surrogate marker salivary alpha-amylase (sAA) from saliva samples (Nater & Rohleder, 2009). The activation of the SAM-axis also induces a cardiovascular reactivity, which can be characterized by measuring changes in for example systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial blood pressure (MAP), or HR (Arza et al., 2019; Bali & Jaggi, 2015; Chida & Hamer, 2008). Similar to EPI and NE, these SAM-related parameters peak during stress induction or immediately after stress cessation and quickly return to baseline levels within 5 to 20 min after stress cessation (Walther & Wirtz, 2023).

Hypothalamic-Pituitary-Adrenal Axis and the Hormone Cortisol

The second arm of the stress system, the HPA-axis, coordinates the stress response along with circadian processes (Russell & Lightman, 2019). Its end-product, the steroid hormone cortisol, is secreted in response to acute stress and also in a circadian pattern (Russell & Lightman, 2019). The HPA-axis reactivity to acute stress is slower than the activation of the

SAM-axis, but it results in an enhanced and prolonged response (Godoy et al., 2018). Accordingly, it has been suggested that the initial response of the SAM-axis is adjusted by the HPA-axis and its end product cortisol (Rohleder, 2019).

When facing stress, networks including direct and indirect pathways from brainstem and limbic system structures stimulate the parvocellular division of the PVN of the hypothalamus (de Kloet et al., 2005; Dedovic et al., 2009; Herman et al., 2003). The parvocellular neurons in turn produce corticotropin-releasing hormone (CRH) and the peptide (arginine) vasopressin (AVP; de Kloet et al., 2005; Dedovic et al., 2009; Herman et al., 2003). CRH and AVP are released into the hypophyseal portal system and reach the anterior pituitary where particularly CRH stimulates the release of the adrenocorticotropic hormone (ACTH) into the systemic circulation (Charmandari et al., 2005; Russell & Lightman, 2019). ACTH acts on the zona fasciculata of the adrenal cortex leading to the synthesis and release of cortisol (Ulrich-Lai & Herman, 2009). Cortisol levels in blood and saliva rise within minutes after the onset of a stressful stimulus, peaking 10 to 30 min after cessation of short acute stress induction and returning to baseline after approximately 60 min (de Kloet et al., 2005; Foley & Kirschbaum, 2010; Kirschbaum & Hellhammer, 1994).

In addition to its adaptive stress response, cortisol is secreted under basal conditions following a specific circadian rhythm (Engeland & Arnhold, 2005; Russell & Lightman, 2019). The basal HPA-axis activity is largely mediated by indirect pathways of the suprachiasmatic nucleus to the PVN, although further mechanisms of circadian control have been identified as well (Agorastos et al., 2020; Engeland & Arnhold, 2005; Russell & Lightman, 2019). The basal release of cortisol occurs in a pulsatile manner, with approximately 10 to 15 secretion phases occurring within a 24-hr period, with the highest secretion in the early morning (Stone et al., 2001). Accordingly, the circadian pattern of cortisol secretion encompasses the cortisol awakening response (CAR), which is a marked increase of cortisol within 30 to 45 min after

awakening (Pruessner et al., 1997; Stalder et al., 2016; Stalder et al., 2022). After this morning peak, cortisol secretion steadily decreases throughout the day reaching the lowest levels at midnight, which is referred to as slope (Ross et al., 2014; Stone et al., 2001). Both basal and stress-induced cortisol release is autoregulated by a negative feedback loop that inhibits HPA-axis activity by acting on the pituitary, the PVN, and limbic system structures (Dedovic et al., 2009; Russell & Lightman, 2019).

The basal HPA-axis activity, and thus the circadian pattern of cortisol secretion, is modulated by several factors, including genetics, sex hormones, age, environmental stressors (Russell & Lightman, 2019), and health conditions (e.g., Wirtz et al., 2007). Similarly, the stress response and the HPA-axis reactivity to acute stressors are influenced by varying factors, such as past experiences of an individual, the genetic make-up, psychological and demographic variables (Foley & Kirschbaum, 2010; Kudielka et al., 2009; McEwen & Stellar, 1993), as well as health condition (e.g., Walther & Wirtz, 2023). Particularly important to note is that women generally show lower cortisol stress reactions than men, except for women in the luteal phase of their menstrual cycle without oral contraceptive use, who have comparable levels (Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005).

A large proportion of cortisol is bound to transporter proteins, mostly to the corticosteroid-binding globulin, whereas only approximately 5% of the total cortisol in the circulation is unbound and biologically active (Russell & Lightman, 2019). Cortisol reaches its target organs via the circulation (de Kloet et al., 2005) and exerts its effects mostly by binding on mineralocorticoid (MR) and glucocorticoid receptors (GR) which are expressed in many cell types in the human body (Agorastos et al., 2020; Gomez-Sanchez & Gomez-Sanchez, 2014). As the affinity of GR for cortisol is considerably lower than those of MR, GR are occupied when glucocorticoid concentrations increase, e.g., during the circadian peak or in response to stress (Agorastos et al., 2020; Joëls et al., 2017). Cortisol binding to MR or GR

causes both rapid-acting (over minutes) and slow (at least 1 hr) effects (Joëls et al., 2011). The rapid, non-genomic effects of cortisol occur without changing gene expression, notably by acting through membrane receptors (Joëls et al., 2011; Tasker et al., 2006). In contrast, the slow, genomic effects result from modulation of the transcription of several responsive genes leading to changes in the levels of the encoded proteins (Joëls et al., 2011; Joëls et al., 2017). Cortisol is engaged in a wide range of regulatory functions, including the support of cardiovascular, immune, metabolic, and neurobiological homeostasis (Buckingham, 2007; Burford et al., 2017; Sapolsky et al., 2000). Cortisol functions in response to acute stress include an enhanced energy supply, e.g., via effects on the carbohydrate and lipid metabolism, and the protection from potentially harmful inflammatory mediators (Buckingham, 2006, 2007). In addition, as a steroid hormone cortisol can easily pass through the blood-brain barrier, affecting brain structures and neurobiological processes involved in cognition and behavior (Lupien et al., 2009; Sandi, 2013; Sapolsky et al., 2000).

While stress-induced cortisol thus exerts suppressive, stimulating, and preparative actions, basal cortisol levels are suggested to play a permissive role in several homeostatic mechanisms (Munck, 2007; Sapolsky et al., 2000). In line, it has been suggested that basal cortisol secretion contributes to a normal stress response (McEwen, 2019). It has also been associated with the coordination of daily activity, metabolism (McEwen & Karatsoreos, 2015), and physiological dynamics that underlie the circadian clock (Dickmeis, 2009).

Cortisol concentrations can be measured in different organic materials, including urine, hair, blood, and saliva (Kudielka et al., 2012). Cumulative cortisol production is assessed in urine (e.g., 24-hr period; Remer et al., 2008) and hair samples (over months; Russell et al., 2012), while the determination of cortisol in blood and saliva reveals momentary concentrations (e.g., Foley & Kirschbaum, 2010). Since salivary cortisol reflects the free (unbound) biologically active fraction of cortisol, determination from pain-free assessed saliva

samples is often preferred (Kirschbaum & Hellhammer, 1994; Törnhage, 2009). To investigate dynamic changes in cortisol (e.g., its acute stress reactivity or its circadian rhythm), the determination of cortisol concentration in saliva samples, assessed at regular timepoints, is particularly useful (Adam & Kumari, 2009; Kirschbaum & Hellhammer, 1994; Stalder et al., 2022).

Methods for the Investigation of the Biological Stress Response and Stress Effects

The standardized and controlled investigation of the acute biological stress response, including repeated measures of neuroendocrine, autonomic, and cardiac functions, may provide a window into an individual's reaction to stressful demands (Bali & Jaggi, 2015; Chida & Hamer, 2008; Linden et al., 2003). The responses to controlled stress tests may therefore allow for mechanistical insights into potential maladaptive processes as well as into short-term and long-term stress effects (Linden et al., 2003). To induce stress under standardized conditions, a considerable number of different methods have been developed in the field of psychobiological stress research (Bali & Jaggi, 2015). These methods particularly differ with respect to the nature of the stressor (i.e., non-mental or mental) used to induce a stress response (Bali & Jaggi, 2015). Importantly, different types of stressors can vary in the extent of the triggered arousal in the SAM- and HPA-axis respectively (Skoluda et al., 2015). Non-mental stress tests include stress stimulation by a physical stressor (e.g., Cold Pressor Test [CPT]; Lovallo, 1975; Pouwels et al., 2019) or exercise (e.g., Bicycle Ergometer Task; Allgrove et al., 2008; Skoluda et al., 2015). Mental stressors in laboratory settings comprise cognitive tasks, such as arithmetic (e.g., Bali & Jaggi, 2015), color-word interference (Stroop Test; Stroop, 1935), and/or public speaking tasks (Feldman et al., 2004). Moreover, mental stressors often incorporate elements of uncontrollability and social evaluation, which have been shown to induce the largest physiological responses (Dickerson & Kemeny, 2004). Examples of tests

that combine these elements with motivated performance include the Trier Social Stress Test (TSST; Kirschbaum et al., 1993) and the Montreal Imaging Stress Task (MIST; Dedovic et al., 2005). The TSST is a frequently used and well-standardized acute psychosocial stress test, that reliably induces psychosocial stress and the resulting neuroendocrine reactivity (Allen et al., 2014; Dickerson & Kemeny, 2004). The TSST is a 15-min psychosocial stress paradigm comprising three parts of equal length, which are conducted in front of unknown evaluators, a video camera, and a microphone (Bali & Jaggi, 2015; Kirschbaum et al., 1993). After a brief instruction from the principal investigator, the participant is given time to prepare a public speaking task (Bali & Jaggi, 2015; Kirschbaum et al., 1993). This phase is followed by a simulated job interview (i.e., a public speaking task) and an arithmetic task (Bali & Jaggi, 2015; Kirschbaum et al., 1993). The MIST is another standardized stress paradigm, that was developed for functional imaging studies and reliably induces psychophysiological stress responses (Dedovic et al., 2005). The procedure comprises computerized mental arithmetic tasks that need to be solved with (stress condition) or without (control condition) time restriction and varying negative feedback (Dedovic et al., 2005). The experimental procedure also includes a control condition to allow the separate investigation of the effects of stress or the cognitive task respectively (Dedovic et al., 2005).

Short-Term Effects of Acute Stress and HPA-Axis Reactivity

The short-term effects of acute stress, beyond the SAM- and HPA-axis reactivity, encompass changes in a variety of physiological systems (such as the renin-angiotensin-aldosterone system; e.g., Gideon et al., 2021) as well as adaptions on the behavioral and cognitive level that are modulated by the stress system (e.g., Chrousos, 1998; Romero & Butler, 2007). Short-term effects on the physiological level include for example the activation of blood coagulation and fibrinolysis (Hjemdahl & von Känel, 2012; von Känel, 2015; von Känel et al.,

2001), increases in inflammatory markers (Marsland et al., 2017; Steptoe et al., 2007), as well as changes in blood lipid profiles (Stoney, Bausserman, et al., 1999; Stoney & Finney, 2000). These transient changes have been interpreted as evolutionary mechanisms protecting the organism against injury (Segerstrom & Miller, 2004; von Känel, 2015). However, exceeding physiological changes in response to acute stress are considered a direct pathway through which stress affects health in the long term (e.g., Brydon & Steptoe, 2005; McEwen, 1998a; O'Connor et al., 2021). Comparable long-term effects of acute stress (with a focus on blood lipids) are discussed more extensively in the chapter “Long-Term Effects of Acute Stress and HPA-Axis Activity”.

Stress can affect health also indirectly through effects on health behaviors (O'Connor et al., 2021; Steptoe & Kivimäki, 2012). In line, stress can promote several health-relevant behaviors, including increased nicotine, alcohol, and high-caloric food consumption (Cohen et al., 2016; Epel et al., 2018). However, the evidence concerning the impact of acute (psychosocial) stress on behavior (Dantzer, 2016), particularly eating behavior (e.g., Adam & Epel, 2007), is not conclusive, and the mechanisms that underlie eating behavior in response to stress are not yet fully understood. Regarding cognitive consequences of acute stress in the short term, expected adaptions include for example increased alertness and focused attention (Chrousos & Gold, 1992). In contrast, a large body of research suggests that acute stress does not always improve cognition, but may also have detrimental effects (e.g., Calvo & Gutiérrez-García, 2016; Sandi, 2013). This equivocal research highlights the need to further investigate the short-term cognitive and behavioral effects of acute stress and to consider the underlying endocrine mechanisms in terms of HPA-axis reactivity.

Acute Stress and Cognition: Effects on Concentration Performance

Stress has an important impact on most cognitive processes, ranging from attention and memory to decision-making, with either facilitating or impairing effects (Sandi, 2013; Vogel et al., 2016). These stress effects on cognition seem particularly relevant in certain occupational groups where work must often be accomplished under stressful conditions including health professions (LeBlanc, 2009) or the military (Beckner et al., 2021). A cognitive function that is crucial for the success of daily and work-related performance is concentration (Avirame et al., 2022; Fortenbaugh et al., 2017; Moran, 2012). Concentration performance can be affected by internal and external distractions, e.g., stress, that can distract attention away from a focus that is relevant to the task at hand (Moran, 2012). The understanding of the effects of acute psychosocial stress on concentration and the underlying mechanisms may offer indications for adjustments in working or learning conditions, potentially leading to improved performance.

Concentration Performance. Concentration, which is often referred to as sustained attention or vigilance (Valdez et al., 2005), describes the decision "to invest mental effort on what is most important in any given situation" (Moran, 2012, p. 119). In detail, concentration is defined as the ability to stay focused while pursuing a task and to ignore distractions in order to maintain a certain level of performance and thus to control attention throughout an extended period (Moran, 2012; Sörqvist et al., 2016; Unsworth & Robison, 2020). Therefore, the ability to concentrate is considered a basic function of attention, which is vital for higher attentional processes (such as selective or divided attention) as well as for cognition in general (Sarter et al., 2001).

The regulation of concentration includes bottom-up (target-triggered) processing, which is determined by the sensory salience of the stimulus, as well as top-down (knowledge-driven) mechanisms that facilitate the processing of pertinent sensory input (Langner & Eickhoff, 2013; Sarter et al., 2001). Multiple brain regions, including the prefrontal cortex, the

cingulate cortex, the locus coeruleus, and subcortical structures, have been associated with the neural regulation of concentration performance (Huang et al., 2023; Sarter et al., 2001). More recent research approaches do not only focus on specific activated brain areas but also try to understand the neuronal networks that regulate concentration performance (Fortenbaugh et al., 2017). A meta-analysis identified 14 distinct clusters of brain areas to be activated in concentration performance tasks (Langner & Eickhoff, 2013). These clusters partly overlapped with other networks such as the executive control attention networks or the salience network (Fortenbaugh et al., 2017).

Concentration performance is assessed in psychological tests that are designed to initiate cognitive processes related to sustained attention (Huang et al., 2023). Classical assessment methods include continuous performance tasks, where individuals are required to detect and respond to specific target stimuli while ignoring distractors in a pre-defined period of time (Fortenbaugh et al., 2017; Huang et al., 2023). Examples of these tasks are computer-based go/no-go tasks such as the Conners' Continuous Performance Test (Conners & Sitarenios, 2011) and the Sustained Attention to Response Test (Manly & Robertson, 2005), where participants are instructed to respond to any presented stimuli except for a nominated non-target stimulus. It is however important to be aware of the fact that this test design may capture response inhibition in addition to sustained attention (Fortenbaugh et al., 2017). The d2 Test of Attention is an alternative paper-and-pencil method for assessing concentration performance (Bates & Lemay, 2004; Brickenkamp, 1994). In this test, participants need to cancel out specific targets among numerous distractors (Bates & Lemay, 2004; Brickenkamp, 1994). Factors that may affect concentration performance in psychological tests and in general include motivation and expertise (Oken et al., 2006; Sörqvist & Marsh, 2015), the cognitive load of the task (Sörqvist et al., 2016), age (Carriere et al., 2010), as well as somatic disorders,

such as cardiovascular diseases (Jerskey et al., 2009), and mental disorders, such as major depression (Piani et al., 2022).

Effects of Acute Stress on Concentration Performance. As mentioned above, most cognitive operations can be affected by stress (Sandi, 2013). The outcome of these stress effects (whether negative or positive) depends on the investigated cognitive process itself and stressor-related factors, including the severity of the stressor (Sandi, 2013; Shields et al., 2016). Accordingly, it is suggested that stress may influence brain processes in an inverted-U shaped manner as a function of the intensity of the stressor (Sapolsky, 2015). In this regard, the shift from the complete absence of stress to mild stress induces an increase in cognitive performance, relatively mild to moderate levels of stress relate to the highest performances, and the transition from moderate to strong stress is related to performance decrease (Sapolsky, 2015). The assumption of an inverted-U shaped association of stress and cognition is partly reflected by the theoretical approaches explaining stress effects on attention, including concentration. In this regard, the arousal model suggests that an optimal level of physiological arousal is vital for attentional processes (Esterman & Rothlein, 2019). Similarly, the capacity theory postulates that individuals have more attentional resources (such as concentration) during periods of total alertness (Kahneman, 1973; Moran, 2012). The attentional control theory claims further that anxiety, which is a result of threatening circumstances and high stress levels, contributes to an attentional bias to threat-related stimuli, which may lead to negative outcomes (Eysenck et al., 2007). In line, emotional arousal (LeBlanc et al., 2015) and acute (mental) stress (Kohn et al., 2017; Sänger et al., 2014) can impair selective attention, i.e., the ability to focus on task-relevant information while ignoring irrelevant stimuli (Murphy et al., 2016). In contrast, mild acute non-mental stress in terms of a modified CPT has been associated with an improved response speed in a selective attention task (Shields et al., 2019). Together, these results corroborate the hypothesis of the inverted-U shaped association between acute stress and

cognitive processes including attention. Whether this relationship also applies to sustained attention, i.e., concentration, needs to be elucidated. To the best of my knowledge, only four studies investigated the effects of acute psychosocial stress on concentration in samples of young adult men (Scholz et al., 2009; von Dawans et al., 2012) or men and women (Banks et al., 2014; Olver et al., 2015). In more detail, the studies compared concentration performance assessed immediately to 10 min after stress cessation either with the performance of a non-stress control group (Banks et al., 2014; Scholz et al., 2009; von Dawans et al., 2012) or with the baseline performance (i.e., before stress; Olver et al., 2015). In the latter study, concentration performance was additionally assessed approximately 1 hr after stress (Olver et al., 2015). Stress induced by means of the TSST was associated with impaired concentration performance in variations of go/no-go tasks shortly and up to 1 hr post-stress (Olver et al., 2015; Scholz et al., 2009). In contrast, stress induction using variants of common stress tests, i.e., the TSST for groups (TSST-G) or the socially evaluated CPT (sCPT) did not affect concentration performance, neither in a go/no-go task (employing the sCPT; Banks et al., 2014) nor in the d2 Test of Attention (employing the TSST-G; von Dawans et al., 2012). With regard to the inverted-U shaped association between stress and cognition, it is not surprising that a strong stressor such as the TSST has a negative influence on concentration performance. It remains to be elucidated why other psychosocial stressors such as the group-TSST and the sCPT did not have an effect in this regard. The investigation of the physiological stress reactivity, in terms of stress-induced cortisol increases, may offer some clarification. It seems likely that the different stress tests differed with respect to the induced physiological stress reactivity (e.g., Skoluda et al., 2015). Given the proposed moderating role of the HPA-axis (i.e., cortisol) reactivity regarding acute stress effects on cognitive performance (Shields et al., 2015; Shields et al., 2016), stress reactivity differences in cortisol may be an explanation for the observed results.

Effects of Stress-Induced Cortisol Increases. Evidence from patients with elevated cortisol levels (e.g., as a symptom of Cushing's disease) showing cognitive impairment (Dekkers et al., 2022; Lupien et al., 2007), supports the notion that stress-induced HPA-axis reactivity in terms of cortisol increases might play a mechanistical role underlying the effects of acute stress on cognitive functioning, including concentration. Further, the administration of corticosteroids has been reported to affect cognitive processes in an inverted-U shaped relationship (Lupien & McEwen, 1997). In line with this reasoning, a study in relatively young men found an association between a single administration of 100 mg hydrocortisone and increased error rates in an attentional (Stroop) task assessed 2 hr after the treatment (Hsu et al., 2003).

Evidence regarding the (acute stress-induced) cortisol effects on concentration performance remains unclear. Two studies in young men and women suggested that cortisol changes induced by means of the TSST (Olver et al., 2015) or a modified CPT (Shields et al., 2019) do not affect attentional processes including concentration performance assessed immediately (within 15 min) and 1 hr after the experimental condition. The researchers of the CPT study attributed the absence of an effect to their cortisol sampling protocol, which prevented the assessment of the cortisol peak after the stress induction (Shields et al., 2019). In contrast to the other recorded stress-reactive parameters in their study, Olver et al. (2015) did not observe increases in cortisol after the TSST, which they attributed to the relatively high cortisol baseline levels of their participants. The absence of stress-induced increases in cortisol may therefore account for the missing association with cognitive performance (Olver et al., 2015). Moreover, in both studies, the previously mentioned sex differences regarding the cortisol stress reactivity (Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005) were not controlled sufficiently, which might also have led to a bias in the

results. The effects of acute psychosocial stress-induced cortisol increase—notably without potentially confounding effects—need therefore further elucidation.

Acute Stress and Behavior: Effects on Eating Behavior

Addressing the increasing prevalence of overweight and obesity (Chooi et al., 2019), eating behavior has gained more relevance in public health research. An unhealthy diet, overweight, and obesity lead to a higher risk of adverse health outcomes, including diabetes mellitus and cardiovascular diseases (e.g., Forouzanfar et al., 2015; Guh et al., 2009). The high prevalence of obesity, along with the substantial burden for the affected individual and the economic costs to societies, emphasizes the importance of understanding the mechanisms that regulate hunger and satiety (Heisler & Lam, 2017; Theilade et al., 2021; Tremmel et al., 2017). Stress has repeatedly been discussed as a risk factor for overweight and obesity (Dallman, 2010; Sinha & Jastreboff, 2013; Tomiyama, 2019). As altered neuro-hormonal homeostasis is one of the most significant factors contributing to obesity (Theilade et al., 2021), understanding the endocrine mechanisms underlying eating after stress, notably in healthy individuals, could be of particular importance.

Eating Behavior. The term eating behavior broadly encompasses different nutrition-related aspects such as food choice, food motives, dieting, and eating disorders (LaCaille, 2013). The regulation of appetite, satiety, and food intake is under the control of the neuro-endocrine system which involves a great number of hormones engaging with specific CNS structures (Alhabeeb et al., 2021; Heisler & Lam, 2017; Theilade et al., 2021). Among these, the hypothalamus and particularly the arcuate nucleus (ARC) play a crucial role in the regulation of appetite and food intake (e.g., Alhabeeb et al., 2021; Huda et al., 2006; Sam et al., 2012). The ARC is located near the median eminence, which allows the integration of endocrine and external signals (Heisler & Lam, 2017; Sam et al., 2012). The ARC comprises

orexigenic and anorexigenic neurons, which are either inhibited or stimulated by (gut) hormones and food intake (e.g., Alhabeeb et al., 2021; Huda et al., 2006; Sam et al., 2012). A further vital brain structure for the regulation of appetite and food intake is the nucleus of tractus solitarius (NTS) of the brainstem (Alhabeeb et al., 2021; Heisler & Lam, 2017; Sam et al., 2012). The NTS is innervated by the vagus nerve, allowing a reciprocal connection between the gastrointestinal tract and the brain (Alhabeeb et al., 2021; Sam et al., 2012). More specifically, the receptors on the local vagal afferent nerve endings can interact, among other signals, with gut hormones (Alhabeeb et al., 2021). In addition to the homeostatic regulation of eating, hedonic eating, i.e., eating for pleasure, is controlled by the mesolimbic reward system (Alhabeeb et al., 2021; Koop & Oster, 2022). Notably, hedonic signals can outweigh homeostatic satiety signals, potentially leading to overconsumption (Alhabeeb et al., 2021; Koop & Oster, 2022). Most gut hormones that contribute to the endocrine regulation of appetite and feeding behavior, derive from the pancreas, adipose tissue, and gastrointestinal tract and are secreted in response to meal ingestion (Theilade et al., 2021). These gut hormones can affect hunger (e.g., ghrelin) and satiety (e.g., leptin; Huda et al., 2006; Theilade et al., 2021; for more details see also Murphy & Bloom, 2006). A well-known representative is the satiety hormone cholecystokinin (CCK), which is released from small intestinal endocrine I cells in response to fat- and protein-rich meal intake (Miller et al., 2021; Rehfeld, 2017). In detail, CCK levels increase within about 10 min after eating and remain elevated more than 3 hr after eating (Burton-Freeman et al., 2004; Liddle et al., 1985). Alongside its diverse functions (e.g., contributing to various digestion processes), CCK is suggested to reduce food intake and is associated with the perception of fullness and satiety (Ballinger & Clark, 1994; Rehfeld, 2017), via acting on receptors of the vagal nerve (Alhabeeb et al., 2021; Murphy & Bloom, 2006).

Paradigms to assess eating behavior in experimental (stress) research include the assessment of the amount of food consumed for example in the context of a standardized ad

libitum buffet (e.g., Blundell et al., 2010; Kistenmacher et al., 2018) or a bogus taste test (Robinson et al., 2017). In the latter participants are required to taste and rate one or several food items, while the outcome of interest is actually the amount of consumed food (Robinson et al., 2017). Further methods to analyze food intake patterns comprise the assessment of chewing sounds (Päßler et al., 2012), subjective ratings of hunger (e.g., van Strien et al., 2012) or appetite for example through visual analog scales (e.g., Herhaus et al., 2020) as well as food preference assessed by the choice between a range of different standardized foods (e.g., Zellner et al., 2006).

In addition to physiological processes, several other factors have been identified that can affect eating behavior (LaCaille, 2013). These factors include psychological processes (e.g., self-regulation), the social and cultural environment of an individual, as well as physical factors such as the availability of foods (LaCaille, 2013). Moreover, eating behavior can vary as a function of mental and physical health (Quick et al., 2013; Simmons et al., 2016). A further important factor that may account for differences in eating behavior is sex as women generally show healthier eating habits than men (de Castro & Kreitzman, 1985; Rolls et al., 1991; Wardle et al., 2004; Westenhoefer, 2005). In addition, men and women seem to differ in their physiology of eating (for review see Asarian & Geary, 2013) including differences in gut hormone concentrations such as in CCK (Burton-Freeman et al., 2004; Nolan et al., 2003).

Effects of Acute Stress on Eating Behavior. Concerning stress effects on eating and eating-related parameters, most studies reported an association of chronic stress with obesity risk (e.g., Brunner et al., 2007). Indeed, chronic stress has repeatedly been linked to a higher body mass index (BMI; Brunner et al., 2007; Cotter & Kelly, 2018) and increases in waist circumference (Brunner et al., 2007; Cotter & Kelly, 2018; Ishizaki et al., 2008) as well as with a higher probability of gaining weight (Chao et al., 2017; Serlachius et al., 2007). Moreover, chronic stress is suggested to contribute to an increased consumption of high-caloric and

palatable food (Newman et al., 2007; Ng & Jeffery, 2003; O'Connor et al., 2008). However, there have also been reports that stress can have an inhibiting effect on food intake (e.g., Stone & Brownell, 1994). Focusing on the effects of acute stress on eating-related measures in healthy individuals may help to shed light on the processes through which stress affects obesity risk.

Regarding acute stress effects on the amount of consumed food, several studies came to rather inconclusive results. Most researchers did not find differences between conditions of acute psychosocial or non-mental (only in Geliebter et al., 2012) stress and a respective non-stress control condition in different study samples; i.e., healthy men and women (Herhaus et al., 2018; Oliver et al., 2000; Petrowski et al., 2014); obese men and women (Herhaus et al., 2018); healthy women (Klatzkin et al., 2018; Raspopow et al., 2014); healthy to obese women (Appelhans et al., 2010; Epel et al., 2001; Tryon et al., 2013); and severely obese women (Geliebter et al., 2012). Compared to a control condition, a higher food intake was however observed in normal and overweight men and women following a mental arithmetic task (Rutters et al., 2009) and in normal weight men after a modified version of the TSST (Kistenmacher et al., 2018). In contrast, another research group found obese and normal weight women to eat less food after the TSST than after a control condition (Kiessl & Laessle, 2016; Kiessl & Laessle, 2017).

Regarding the impact of acute stress on other eating-related parameters, other researchers observed a higher chewing frequency after acute stress induction in terms of the TSST in male and female participants with normal to obese BMI ranges (Herhaus et al., 2018; Petrowski et al., 2014). One study in women described a preference for high-fat instead of low-fat foods (Zellner et al., 2006) while another study in both sexes did not find effects on food preference (Hyldelund et al., 2022) after a stressful cognitive task. As for the subjective perception of hunger or appetite, most studies in healthy normal weight up to obese women

and/or men reported lower appetite (Herhaus et al., 2020; Nakamura et al., 2020; Petrowski et al., 2014), hunger (van Strien et al., 2014), and desire to eat (Geliebter et al., 2012) after acute psychosocial (i.e., speech tasks including the TSST) or CPT-induced stress as compared to a control condition. Two other studies found however no effects on satiety perception (Kiessl & Laessle, 2016) and appetite (Oliver et al., 2000) after acute psychosocial stress. Regarding stress effects on appetite and satiety hormones, ghrelin (Bouillon-Minois et al., 2021; McKay et al., 2021) and leptin (Tomiyama et al., 2012) were reported to rise in reaction to the TSST. CCK reactivity to a standardized acute mental stress test has not yet been investigated so far.

Regarding potential sex differences in food intake after stress, research is similarly inconclusive. Three studies compared food intake after acute mental stress and respective control conditions between men and women. Grunberg and Straub (1992) reported lower food intake after stress in men, while Oliver et al. (2000) found a higher food intake in men both following stress and after a non-stressful condition. In the third study, no sex-related differences in eating after stress were found (Rutters et al., 2009). Whether men and women differ in hunger and the potential reactivity of appetite and satiety hormones after acute stress requires further research.

Taken together, the effects of acute stress on eating behavior have been addressed in many studies using relatively similar study designs and samples. These studies, however, do not allow for any general conclusions about the effects of acute stress on eating behavior. Here, the underlying endocrine mechanisms might contribute to a clearer understanding of the effects of stress.

Effects of Stress-Induced Cortisol Increases. Since glucocorticoids have been suggested to have an important effect on eating behavior (Dallman et al., 2004), and since the stress and feeding systems partly overlap (Maniam & Morris, 2012), stress-induced cortisol increases may be a potential mediator underlying the psychobiological mechanisms of eating

after stress. Cortisol is thought to affect eating after stress by various complex mechanisms notably in interaction with orexigenic and anorexigenic neuropeptides of the ARC on the one hand and with peripheral signals such as appetite and satiety hormones on the other hand (Maniam & Morris, 2012). In line, cortisol excess has been suggested to impair hormonal appetite and satiety signaling and consequently eating control (for review see Kuckuck et al., 2023).

However, several studies comparing food intake after standardized psychosocial stress induction in cortisol high vs. cortisol low stress reactors came to rather contradictory results: Healthy female cortisol high reactors ate more after the TSST as compared to cortisol low reactors (Epel et al., 2001). Further, the researchers detected a positive linear association between food intake and cortisol change after stress (Epel et al., 2001). Two similar investigations could not find TSST-induced cortisol effects in study samples of lean women (Appelhans et al., 2010) and normal weight men and women (Herhaus et al., 2020). Two other studies, however, observed a higher food intake in female cortisol low reactors to the TSST (Klatzkin et al., 2018; Tryon et al., 2013). With respect to investigations in obese participants, one study found higher TSST-induced cortisol levels to be associated with decreased food consumption in obese women (Appelhans et al., 2010). Another investigation in obese men and women reported higher food consumption after stress (i.e., TSST) in cortisol high reactors compared to those with a lower cortisol reaction (Herhaus et al., 2020). Further, plasma cortisol reactivity to the CPT was not associated with subsequent food consumption in severely obese women (Geliebter et al., 2012).

Considering the subjective perception of appetite, two studies did not find associations with TSST-induced cortisol in male and female normal weight up to obese participants (Herhaus et al., 2020; Petrowski et al., 2014). In contrast, cortisol peak levels after the CPT were positively correlated with hunger in overweight women (Geliebter et al., 2013).

The evidence on possible associations between stress-induced cortisol levels and gut hormones related to hunger and satiety is not unequivocal. Whereas one study reported a positive relation between changes in ghrelin and cortisol following the TSST (Rouach et al., 2007), TSST-induced leptin reactivity was not associated with cortisol in another investigation (Tomiyama et al., 2012). Whether stress-induced cortisol levels are associated with CCK still needs to be elucidated. Taken together, evidence regarding the role of the HPA-axis reactivity on eating-related parameters is not unequivocal and warrants further elucidation.

Long-Term Effects of Acute Stress and HPA-Axis Activity

The frequent, prolonged, and/or inadequate response of stress mediators is associated with the long-term dysregulation of other physiological systems, thereby increasing the risk for a wide range of diseases (Chrousos, 1998; Cohen et al., 2007; McEwen, 1998b; McEwen & Seeman, 1999; McEwen & Stellar, 1993; O'Connor et al., 2021). Indeed, a large number of studies prove the negative effects of stress on health (e.g., Guidi et al., 2021; Turner et al., 2020). Over the past decades, stress has been recognized as an increasingly important risk factor for cardiovascular diseases and particularly for CHD (e.g., Black & Garbutt, 2002; Brotman et al., 2007; Kivimäki & Steptoe, 2018; Lagraauw et al., 2015; Rosengren et al., 2004; Steptoe & Kivimäki, 2012) but the underlying mechanisms are still not completely understood.

Coronary Heart Disease and Atherosclerosis

CVD are a major cause of premature mortality and disability producing immense health and economic burdens globally (e.g., Flora & Nayak, 2019; Mensah et al., 2023; Timmis et al., 2022; Tsao et al., 2023). An important cardiovascular disease is CHD (Tsao et al., 2023), with a prevalence of approximately 5,430 per 100,000 in 2022 in central Europe (Mensah et al.,

2023) and accounting for about half of all disability-adjusted life years (DALYs) due to CVD (Vardas et al., 2022).

CHD is caused by the progressive narrowing of the coronary arteries limiting the supply of oxygenated blood to the cardiac muscle (Flora & Nayak, 2019; Jensen et al., 2020; Wirtz & von Känel, 2017). The most frequent process underlying CHD is atherosclerosis (Falk, 2006), which is defined as the deposition of fatty and fibrous components in the intima, the innermost layer of arteries (Hansson & Hermansson, 2011; Libby et al., 2019). It has been suggested that high circulating blood lipid levels promote the initiation of the pathophysiology of atherosclerosis (Hansson & Hermansson, 2011; Leon & Bronas, 2009). Atherosclerosis begins with endothelial dysfunction, including increased permeability of the endothelium that is accompanied by the retention of low-density lipoprotein cholesterol (LDL-C; Jebari-Benslaiman et al., 2022; Ross, 1999). LDL-C accumulates in the intima, where (mostly) oxidative modifications cause it to become pro-inflammatory and immunogenic (Hansson & Hermansson, 2011; Libby et al., 2019). Modified lipids can activate endothelial cells and macrophages, triggering the production of adhesion molecules and cytokines (Hansson & Hermansson, 2011). The latter attract immune cells including monocytes and T-lymphocytes (Hansson & Hermansson, 2011). Monocytes migrate into the intima and mature into macrophages (Hansson & Hermansson, 2011; Libby et al., 2019). These macrophages ingest modified LDL-C particles and transform into lipid-laden foam cells, which are specific for atherosclerotic lesions (Hansson & Hermansson, 2011; Libby et al., 2019). The foam cells further stimulate local inflammation and release inflammatory mediators including pro-inflammatory cytokines (Hansson, 2005; Hansson & Hermansson, 2011; Libby, 2012; Wirtz & von Känel, 2017). In addition, T-lymphocytes infiltrate the intima and modulate the functions of other involved cells, such as innate immune, endothelial, and smooth muscle cells (SMCs; Libby et al., 2019). Early atherosclerotic lesions—the fatty streaks—are composed of

foam cells and T-lymphocytes (Ross, 1999; Wirtz & von Känel, 2017). These lesions together with mediators elaborated by the accumulating leukocytes attract SMCs toward the fatty streak in the intima (Libby et al., 2019; Libby & Theroux, 2005; Malakar et al., 2019). The SMCs start to produce extracellular matrix molecules, including collagen and proteoglycans (Libby et al., 2019; Malakar et al., 2019). This process contributes to the formation of atherosclerotic plaques and the thickening of the intimal layer and leads to the progression of atherosclerotic lesions (Libby et al., 2019; Malakar et al., 2019) which is commonly accompanied by cell death (Libby, 2021; Libby et al., 2019). At this stage, atherosclerotic plaques are characterized by the presence of a necrotic core, which is conceived as a depot of cellular debris and lipids (Jebari-Benslaiman et al., 2022; Libby, 2012). A fibrous cap forms a coat over the necrotic core (Jebari-Benslaiman et al., 2022; Libby et al., 2019; Wirtz & von Känel, 2017). Activated T-lymphocytes, mast cells, and macrophages enter the so-called shoulder regions of this formation and secrete (among others) pro-inflammatory mediators (Hansson & Hermansson, 2011; Wirtz & von Känel, 2017). The continued growth of the atherosclerotic plaque results in the development of flow-limiting lesions, which narrow the arterial lumen and promote ischemia in the adjacent tissues (Hansson & Hermansson, 2011; Libby et al., 2019; Wirtz & von Känel, 2017). Moreover, structural weakening of the fibrous cap leads to an increased susceptibility to plaque rupture, which in turn can trigger acute thrombosis (Libby et al., 2019). The thrombotic response can either block the lumen or detach in the form of an embolus causing ischemia in other tissues (Hansson & Hermansson, 2011; Spronk et al., 2004; Wirtz & von Känel, 2017).

CHD is thus defined as a chronic disease that can, at any time, shift to an unstable state generally caused by an acute atherothrombotic event following plaque rupture (Jensen et al., 2020). Due to the dynamic progression of atherosclerosis, clinical manifestations of CHD can be classified as acute (e.g., unstable angina pectoris, acute myocardial infarction [MI]) or

chronic coronary syndromes (e.g., stable angina pectoris, new onset of heart failure; Byrne et al., 2023; Knuuti et al., 2019). The probability of developing atherosclerosis and incident CHD is significantly determined by specific risk factors. These include traditional (i.e., non-modifiable and modifiable) CHD risk factors (e.g., Flora & Nayak, 2019; Hajar, 2017) as well as novel markers such as the intermediate biological CHD risk factors (e.g., Ridker et al., 2018; Wirtz & von Känel, 2017).

Risk Factors for CHD and Atherosclerosis

Intermediate Biological Risk Factors. A number of biological markers associated with the process of atherosclerosis are independent predictors of CHD allowing insights into the underlying mechanisms (Aday & Ridker, 2019; Ridker et al., 2001; Rodondi et al., 2010). These intermediate biological risk factors for CHD comprise blood lipid levels, as well as different markers of coagulation and inflammation (Aday & Ridker, 2019; Ridker et al., 2000; Ridker et al., 2001; Willeit et al., 2013).

Elevated circulating blood lipid levels, which are attributable to both genetic origins and lifestyle (therefore also conceived as a traditional modifiable risk marker; see below), promote atherosclerotic processes and are considered a key risk factor for CHD (Hansson & Hermansson, 2011; Jebari-Benslaiman et al., 2022; Leon & Bronas, 2009; Tomkin & Owens, 2012). Exposure of the intima to above-ideal LDL-C levels has been associated with atherosclerosis (Libby, 2021). Similarly, elevated levels of total cholesterol (TC), a surrogate measure of LDL-C, and triglycerides (TG) are related to CHD risk (Leon & Bronas, 2009). High-density lipoprotein cholesterol (HDL-C) has been suggested to fulfill anti-atherosclerotic effects (Libby, 2021) as it contributes for example to the removal of cholesterol in cells (Leon & Bronas, 2009). Therefore, HDL-C may have protective effects and low concentrations are considered atherogenic (Leon & Bronas, 2009; Libby, 2021). Chronically elevated fasting

LDL-C, TC and TG levels, together with decreased levels of HDL-C are referred to as dyslipidemia (also commonly described as hyperlipidemia; Genest & Libby, 2018; Leon & Bronas, 2009). The ratio TC/HDL-C has been proposed as a suitable index for the magnitude of dyslipidemia that has a strong predictive value for CHD risk (Calling et al., 2021; Lemieux et al., 2001).

As described above, cellular and molecular inflammation pathways are central elements of the process of atherosclerosis (Aday & Ridker, 2019; Libby, 2012). In fact, circulating biomarkers of inflammation, including pro-inflammatory cytokines (Yudkin et al., 2000) and acute-phase proteins (Libby & Ridker, 2004), have been related to atherosclerosis. Proinflammatory cytokines, such as tumor necrosis factor α (TNF-α) or interleukin-6 (IL-6), are released by different cells, e.g., activated macrophages or foam cells, during the atherosclerotic process (Aday & Ridker, 2019; Galkina & Ley, 2009; Libby, 2002). The cytokine TNF-α fulfills potent pro-inflammatory effects (Kleemann et al., 2008) and triggers, in addition to other cytokines, the production of IL-6 (Hansson, 2005; Hunter & Jones, 2015). Moreover, TNF-α facilitates the binding of LDL-C to the endothelium and SMCs and is involved in the activation of T-lymphocytes and the formation of foam-cells (Ross, 1999). IL-6 contributes to the immune response and acute-phase reaction (Kleemann et al., 2008). IL-6 triggers for example the production of acute-phase proteins such as fibrinogen and C-reactive protein (CRP; Hansson, 2005; Ridker, 2016). Moreover, upstream IL-6 signaling has been suggested to contribute to the formation and destabilization of plaque (Ridker, 2016). The acute-phase protein CRP is produced by hepatocytes (Aday & Ridker, 2019). With respect to its role in atherosclerosis, it has been suggested that CRP can, among others, regulate the LDL-C uptake of macrophages and promote increases in adhesion molecules (Libby & Ridker, 2004; Shrivastava et al., 2015). In line with these findings, TNF-α (Kaptoge et al., 2014; Tuomisto et al., 2006), IL-6 (Danesh et al., 2008; Kaptoge et al., 2014; Ridker et al., 2000), and CRP

(Emerging Risk Factors Collaboration et al., 2010; Rifai & Ridker, 2001) are important predictors of increased risk for CHD and cardiovascular events.

As the underlying process of atherosclerosis includes thrombotic responses, biological markers reflecting activated coagulation and fibrinolysis may be associated with CHD risk (Olie et al., 2018; Willeit et al., 2013). In addition to other hemostatic factors, plasma fibrinogen (Fibrinogen Studies Collaboration et al., 2005; Woodward et al., 1998) and D-dimer (Simes et al., 2018; Soomro et al., 2016; Willeit et al., 2013) are conceived as predictors of future CHD risk. With respect to its atherogenic properties, fibrinogen plays (among other functions) a crucial role in determining the activation of platelets and contributes to the production of adhesion molecules (Surma & Banach, 2021). In addition, fibrinogen participates in the final phase of the clotting process by converting into fibrin, resulting in a blood clot (Surma & Banach, 2021). Increased plasma concentrations of fibrinogen directly activate pro-atherogenic mechanisms that can intensify the progression of atherosclerosis (Kryczka et al., 2021). D-dimer results from the degradation of cross-linked fibrin and can be used as a parameter for hemostatic activation, i.e., hypercoagulability and fibrinolysis, notably before overt thrombosis (Simes et al., 2018; von Känel & Dimsdale, 2003; von Känel et al., 2001; Willeit et al., 2013). A hypercoagulable state, defined as the elevated tendency of the blood to thrombose (Senst et al., 2023), results from an imbalance between procoagulant molecules and anticoagulant factors and can lead to thrombotic lesions in the coronary arteries (von Känel & Dimsdale, 2003).

Non-Modifiable Risk Factors. Non-modifiable risk factors for CHD encompass age, sex, ethnicity, as well as genetic and hereditary factors (Flora & Nayak, 2019). The probability of CHD increases with age (e.g., Knuuti et al., 2019). Aging is accompanied by pathological alterations of cardiovascular tissues and functioning including impaired endothelial function that may promote atherosclerosis (North & Sinclair, 2012; Tyrrell & Goldstein, 2021). With

respect to the non-modifiable risk factor sex, men have been found to show a higher prevalence (e.g., Tsao et al., 2023) and to develop CVD and CHD earlier in life than women (Baker et al., 2003; Maas & Appelman, 2010). Moreover, it has been suggested that men experience substantially more total CHD-caused DALYs as compared to women (Roth et al., 2020). Exposure to estrogens may delay the onset of atherosclerotic diseases in women (Maas & Appelman, 2010). Accordingly, the risk of CVD increases significantly with menopause, which is accompanied by estrogen loss (Aggarwal et al., 2018). Differences between men and women regarding CHD prevalence may however also be explained by other biological processes as well as social and environmental factors (Aggarwal et al., 2018). With respect to ethnicity, differences have been observed in CHD prevalence, with the highest CHD prevalence estimates in American Indian/Alaska Native individuals (Tsao et al., 2023). Moreover, genetics and family history account for CHD risk (Tsao et al., 2023). Accordingly, genome-wide association studies have identified genetic variants associated with increased risk for CHD (e.g., Ripatti et al., 2010; Roberts & Stewart, 2012; Tikkanen et al., 2013). These gene variants have been related to CHD events, the atherosclerotic process, cardiovascular biomarkers and traditional risk factors (Howson et al., 2017; Tsao et al., 2023).

Modifiable Risk Factors for CHD and the Important Role of Hypertension. Modifiable risk factors for CHD can be categorized into health behaviors or lifestyle factors on the one hand and clinical (health) factors on the other hand (Timmis et al., 2022; Tsao et al., 2023; Yusuf et al., 2020). Behavioral risk factors encompass for example smoking (e.g., Gallucci et al., 2020), low physical activity (e.g., Sattelmair et al., 2011), or as already mentioned earlier diet quality and eating behavior (e.g., Bechthold et al., 2019; Shan et al., 2020). These can promote clinical factors, such as overweight and obesity (Malik et al., 2013), type 2 diabetes (Hu et al., 2001; Kolb & Martin, 2017) or elevated long-term blood glucose levels in terms of glycosylated hemoglobin (HbA1c; Jansen et al., 2013), elevated blood lipid

levels (Mensink et al., 2003; Mente et al., 2017), as well as increased BP (Oort et al., 2020; Whelton et al., 2018). These clinical factors are considered to be the leading drivers of CHD-related disease burden (Wang et al., 2021), as they can affect the pathogenesis of atherosclerosis via several pathways, including oxidative stress and inflammation (Katakami, 2018; Kattoor et al., 2017; Lovren et al., 2015).

Chronically elevated BP or hypertension is widely recognized as a major modifiable risk factor for CVD and CHD (e.g., Brouwers et al., 2021; Fuchs & Whelton, 2020; Whelton et al., 2018). Hypertension can affect atherosclerosis on different levels, including the development of arterial stiffness, endothelial dysfunction, as well as plaque formation, growth and rupture (Zaheer et al., 2016). In addition, hypertension is recognized as a leading contributor to the global burden of disease (Forouzanfar et al., 2017; GBD 2017 Risk Factor Collaborators, 2018) with a prevalence of more than 30% in adults worldwide (Zhou et al., 2021). According to the Guidelines of the European Society of Cardiology, hypertension is defined as the chronic elevation of BP with repeated office SBP values of ≥ 140 mmHg and/or DBP values ≥ 90 mmHg (Mancia et al., 2023; Williams et al., 2018). Hypertension can be classified according to office BP ranges, including grade 1 with SBP values of 140–159 mmHg and/or DBP values of 90–99 mmHg, grade 2 with SBP values of 160–179 mmHg and/or DBP values of 100–109 mmHg, and grade 3 with SBP values of ≥ 180 mmHg and/or DBP values of ≥ 110 mmHg (e.g., Mancia et al., 2023). It is however important to note that there is a continuous relationship between BP and cardiovascular risk, starting from an office SBP > 115 mmHg and an office DBP > 75 mmHg (Mancia et al., 2023). For a reliable diagnosis and to exclude the presence of other types of hypertension (e.g., white coat or masked hypertension), repeated office BP measurements are recommended, which should be confirmed by an out-of-office assessment (Mancia et al., 2023; Stergiou et al., 2021; Unger et al., 2020; see also for more details regarding the classification and diagnosis of hypertension). Moreover,

hypertension is classified into a primary (essential) and a secondary form (e.g., Mancia et al., 2023). Most affected individuals are diagnosed with essential hypertension (Carretero & Oparil, 2000; Messerli et al., 2007). Numerous mechanisms (including hemodynamic, neural, vascular, and renal mechanisms as well as the activation of the renin-angiotensin-aldosterone system) are suggested to contribute to essential hypertension (Victor, 2018). However, for affected individuals, an apparent and reversible medical cause for their condition cannot be identified (Carretero & Oparil, 2000; Messerli et al., 2007; Victor, 2018). Secondary hypertension accounts only for 5% to 10% of all hypertensive cases (Rimoldi et al., 2014). Here, elevated BP levels have an identifiable medical cause, such as renovascular disease (Rimoldi et al., 2014; Whelton et al., 2018). To exclude the presence of secondary hypertension, laboratory analysis of certain parameters (e.g., creatinine, sodium) should be part of the diagnosis of essential hypertension (Unger et al., 2020).

It is important to note that metabolic risk factors often occur together (e.g., as metabolic syndrome) and may have mutual effects (Leon & Bronas, 2009; McEvoy et al., 2018). Similarly, hypertensive individuals often present further cardiovascular risk factors including older age, male sex, overweight and obesity, diabetes, dyslipidemia, and unhealthy lifestyle habits (Messerli et al., 2007; Unger et al., 2020; Whelton et al., 2018). With regard to CHD risk, it is particularly precarious that hypertensives frequently show an excess proportion of atherogenic lipoproteins with increased levels of TC, TG, and LDL-C as well as decreased HDL-C (Choudhury et al., 2014; Dalal et al., 2012; Gebrie et al., 2018; Sarwar et al., 2014). This physiological interplay of hypertension and dyslipidemia has been proposed to significantly increase cardiovascular risk (Dalal et al., 2012).

Effects of Acute Stress and HPA-axis Activity on the Development and Progression of CHD

As researchers failed to completely explain incident CHD by the above-described (particularly traditional) risk factors—which together explain up to 75% of the cases—interest in the contributing role of psychosocial factors rose (Beaglehole & Magnus, 2002; Strike & Steptoe, 2004). The findings of the Interheart study confirmed that psychosocial factors are a major contributor to MI, accounting for approximately 33% of the population attributable risk (Yusuf et al., 2004). Relevant psychosocial factors under investigation often include negative affect, personality traits and features of the social environment (von Känel, 2012). One psychosocial factor whose role has extensively been discussed in the context of the pathogenesis of CHD is stress (Kivimäki & Steptoe, 2018; Steptoe & Kivimäki, 2012; Steptoe & Kivimäki, 2013). Accordingly, stress (e.g., work-related strain) has been associated with the development of atherosclerosis (Kamarck et al., 2012; Spencer et al., 2024), with the occurrence of CHD (events; Chandola et al., 2008; Kivimäki et al., 2012; Rosengren et al., 2004), and with risk factors such as hypertension (Liu et al., 2017; Sparrenberger et al., 2009; Spruill, 2010).

Several pathways explaining the association between stress and negative health outcomes have been identified. The stage model of stress and disease combines the knowledge of the different stress research traditions, “wherein events appraised as stressful are viewed as triggering affective states that in turn engender behavioral and biological responses having possible downstream implications for disease” (Cohen et al., 2016, p. 456). Particularly chronic stress is suggested to lead to long-term and persistent changes in physiological and behavioral responses that in turn influence disease susceptibility and progression (Cohen et al., 2007). The allostatic load model provides a suitable framework for understanding the long-term effects of stress and stress mediators on interrelated biological systems that can contribute to CHD risk (Juster et al., 2010; McEwen, 1998b; McEwen & Seeman, 1999). The primary stress mediators

(i.e., cortisol, catecholamines, and cytokines) exert primary effects on a cellular level that can lead to secondary outcomes reflecting the cumulative primary effects (Juster et al., 2010; McEwen, 2003; McEwen & Seeman, 1999). The dysregulation of the primary mediators is compensated by related biological systems that in turn change their own operating ranges (Juster et al., 2010). These secondary outcomes represent a prodromal stage, where metabolic (e.g., blood lipid levels), cardiovascular (e.g., BP), and immune parameters (e.g., CRP) reach subclinical levels (Juster et al., 2010; McEwen & Seeman, 1999). Tertiary outcomes, or allostatic overload, refer to the disordered and diseased endpoints that result from the persistent allostatic load (Juster et al., 2010; McEwen & Seeman, 1999). What is still not fully understood is how physiological responses to stress can become maladaptive and thus promote disease (Musazzi et al., 2017). Studying the long-term effects of acute stress may offer valuable insights into the role of stress in disease development and progression (Musazzi et al., 2017).

Long-Term Effects of Acute Stress. As already described above, stress can affect health indirectly on a behavioral level by inducing changes in health-related behaviors (O'Connor et al., 2021). For example, eating more palatable food to comfort negative emotions related to stress has been linked to an increased likelihood of developing obesity, which can in turn promote CHD risk (Hill et al., 2022; Tomiyama, 2019). The consequences of stress with respect to the onset and gradual progression of CHD can thus be partially explained by its indirect impact on health behavior (Chandola et al., 2008).

The stress-induced biological responses provide a further important and direct pathway through which stress affects health (Cohen et al., 2007; McEwen & Seeman, 1999; O'Connor et al., 2021). Acute stress induces short-term adaptive responses in the cardiovascular, immune, and metabolic systems (e.g., McEwen & Seeman, 1999). These adaptions include increases in the intermediate biological CHD risk factors related to coagulation and fibrinolysis (von Känel, 2015; von Känel et al., 2001), inflammation (Marsland et al., 2017; Steptoe et al., 2007) as well

as in atherogenic blood lipids (Stoney, Bausserman, et al., 1999; Stoney & Finney, 2000; Wirtz et al., 2009). Since acute stress has been associated with transient endothelial dysfunction (Ghiadoni et al., 2000), the blood lipid stress reactivity could be of particular clinical relevance for the pathophysiology of atherosclerosis. The blood lipid stress reactivity is maintained as long as the stressor is present (Black, 2002), and disappears after stress cessation (Marcondes et al., 2012; Stoney, Niaura, et al., 1999). However, a study in healthy individuals reported that the extent of the blood lipid stress reactivity is associated with fasting lipid levels after a period of 3 years (Steptoe & Brydon, 2005). Whether the blood lipid stress reactivity also has predictive value for other intermediate biological risk factors related to CHD remains to be elucidated.

With respect to the mechanisms that underlie the blood lipid reactivity to acute stress, EPI promotes lipolysis leading to an increase in circulating free fatty acids (Arner, 2005; Stoney, 2017). NE has been suggested to decrease the activity of the enzyme lipoprotein lipase, which prevents the clearance of TG (Stoney, 2017). Stress-induced cortisol increases contribute to the lipolytic actions of catecholamines (Marcondes et al., 2012). Cortisol inhibits the secretion of insulin, which blocks the regulation of TG and delays the breakdown of LDL-C (Stoney, 2017). Moreover, acute stress induces hemoconcentration (i.e., a temporary decrease of plasma volume resulting in a passive increase of blood constituents), which can partly account for the blood lipid increases (Bachen et al., 2002; Stoney, 2017).

Individuals with a comparably high cardiovascular (Carroll et al., 2011; Chida & Steptoe, 2010; Krantz & Manuck, 1984; Treiber et al., 2003) and/or endocrine stress reactivity (Hamer et al., 2012; Hamer & Steptoe, 2012) are particularly susceptible for CVD. Similarly, and in line with the previously mentioned study (Steptoe & Brydon, 2005), individuals with an elevated blood lipid stress reactivity may have an increased long-term risk (Stoney, 2017). In this regard, it is important to note that hypertensives show stress hyperreactivity in interrelated

stress-reactive systems, which further emphasizes their elevated CHD risk (e.g., Walther & Wirtz, 2023). Similarly, hypertensive participants displayed an increased blood lipid response to acute stress as compared to normotensives (Wirtz et al., 2009). As excess LDL-C may be retained in the intima promoting atherosclerosis (Hansson, 2005; Libby et al., 2019), it needs to be elucidated whether the blood lipid stress (hyper)reactivity is a pathway explaining the increased CHD risk of hypertensives.

Long-Term Effects of the HPA-Axis Activity. As described earlier, the stress system is tightly connected with the circadian system (Agorastos et al., 2020; Koch et al., 2017). Effective communication between both systems is crucial for maintaining homeostasis and facilitating adaptation (Agorastos et al., 2020). Similarly, evidence suggests that not only its stress response but also the basal regulation of the HPA-axis is associated with a wide range of medical conditions and that it may be indicative of allostatic load (Agorastos et al., 2020; Karlamangla et al., 2022; Koch et al., 2017). In this regard, the basal HPA-axis activity has been suggested to represent an intermediate pathway by which psychosocial stress promotes CHD risk (Chandola et al., 2008; Knight et al., 2021).

As cortisol is tightly connected with the regulation of the metabolic, immune, and cardiovascular systems (Sapolsky et al., 2000), the dysregulation of the diurnal HPA-axis activity may interfere with their functioning (O'Connor et al., 2021). Accordingly, alterations of the cortisol diurnal rhythm, particularly flatter diurnal profiles, have been associated with a wide variety of negative health outcomes (Adam et al., 2017; O'Connor et al., 2021), including increased CHD risk (Matthews et al., 2006). Similarly, cross-sectional evidence points to associations between alterations in the basal HPA-axis activity and intermediate biological risk factors, such as inflammation (Knight et al., 2021; Piazza et al., 2018) or dyslipidemia (Dai et al., 2021; Rosmond & Björntorp, 2000). Consistent with these findings, individuals with an increased CHD risk, such as hypertensives (e.g., Wirtz et al., 2007), but also CVD-patients

(e.g., Vreeburg et al., 2009) have been reported to show an altered basal HPA-axis activity as compared to healthy individuals with normal BP ranges. These alterations include for example a flatter CAR (Vreeburg et al., 2009; Wirtz et al., 2007) or elevated evening cortisol levels (Kidambi et al., 2007; Nijm et al., 2007). Moreover, elevated cortisol levels in the morning have been observed in hypertensive compared to normotensive individuals (Kidambi et al., 2007) and a flatter slope has been associated with coronary artery calcification (Matthews et al., 2006). To the best of my knowledge, no study to date compared different elements of the basal HPA-axis activity (i.e., morning and evening as well as total daily cortisol levels, CAR, and diurnal slopes) in healthy normotensives, hypertensive individuals, and CHD-patients. Moreover, alterations in the basal HPA-axis activity predicted prospective changes in subclinical CVD-markers, in terms flow-mediated artery dilation (Violanti et al., 2018), as well as future CHD events and mortality (e.g., Hammer et al., 2016; Ronaldson et al., 2015). However, the underlying mechanisms (with respect to effects on intermediate biological CHD risk factors) remain to be elucidated.

Outline and Research Aims

The adaption to a stressor encompasses physiological, cognitive-affective, and behavioral reactions (Chrousos & Gold, 1992; Epel et al., 2018; McEwen, 1998a). The mediators of the stress system orchestrate these short-term effects on different levels of the organism that can however lead to long-term alterations affecting health (Chrousos, 1998; McEwen & Stellar, 1993). A significant body of research has been devoted to the biological mechanisms underlying the immediate and future consequences of stress. However, with respect to the above-described research, many aspects remain to be elucidated. The overall aim of the present thesis was therefore to shed light on the short-term and long-term effects of acute

stress and HPA-axis (re)activity on different levels and to uncover the underlying biological mechanisms.

Short-Term Effects of Acute Stress and HPA-Axis Reactivity in Healthy Individuals

As it is widely agreed that preclinical research is required to fully comprehend the impact of stress and stress mechanisms on the human body (Allen et al., 2014), the first aim of this thesis was to elucidate the endocrine mechanisms underlying acute psychosocial stress effects on a cognitive and behavioral level in healthy individuals. Concentration performance and eating behavior are two important aspects of daily life (Chooi et al., 2019; Moran, 2012). Potential effects of acute stress on these two factors are of particular relevance for managing everyday tasks and professional responsibilities or for health respectively. Investigating the underlying endocrine mechanisms could enhance our understanding of the effects of acute stress on these two aspects, with possible implications for everyday and professional performance, as well as for health and disease prevention. The short-term adaptive responses to stress are assumed to protect the organism (Dhabhar, 2018; McEwen & Seeman, 1999), however, the above-mentioned studies came to inconsistent findings with respect to acute stress effects and HPA-axis reactivity on concentration performance and eating behavior. In the first two (cross-sectional) studies of the thesis, my colleagues and I therefore investigated the short-term effects of acute stress and stress-induced cortisol increases on concentration performance and eating behavior after acute psychosocial stress respectively. To induce (salivary) cortisol stress responses, we applied the TSST in both studies as it reliably induces psychosocial stress and the resulting neuroendocrine reactivity (Dickerson & Kemeny, 2004).

In more detail, the *first study* focused on acute stress effects on concentration performance. The study aimed to close the above-described methodologic issues of previous research projects regarding study design (Banks et al., 2014; Olver et al., 2015; Scholz et al.,

2009; von Dawans et al., 2012) and cortisol stress reactivity assessment (Olver et al., 2015; Shields et al., 2019). We therefore tested whether male participants of a stress group would differ from a control group in concentration performance changes, assessed before and 30 min after stress induction and a control task respectively. To shed light on the potential underlying mechanisms, salivary cortisol was repeatedly assessed before and up to 60 min after each condition. The further assessment comprised sAA, state anxiety, and anticipatory cognitive stress appraisal.

The *second study* focused on the endocrine mechanisms modulating eating after acute psychosocial stress in healthy men and women. More specifically, we investigated whether stress-induced cortisol release relates to food intake in a taste test starting 15 min after stress cessation, as well as to the potential reactivity of CCK and sensation of hunger before, during, and after the taste test. A further aim was to uncover possible sex differences in eating after stress, as men and women generally differ in their eating habits (de Castro & Kreitzman, 1985; Rolls et al., 1991; Wardle et al., 2004; Westenhoefer, 2005) and their obesity risk (Chooi et al., 2019). The study procedure comprised the repeated assessment of cortisol and the satiety hormone CCK as well as feelings of hunger before and up to 60 min after the TSST.

Long-Term Effects of Acute Stress and Basal HPA-Axis Activity in Healthy and Hypertensive Individuals as well as CHD-Patients

A further aim of the thesis was to gain new mechanistic insights regarding the role of acute stress and basal HPA-axis activity in the pathogenesis of atherosclerosis in healthy and essential hypertensive participants as well as CHD-patients. As previously described, blood lipids are crucial for the initiation of atherosclerosis (e.g., Jebari-Benslaiman et al., 2022; Libby, 2021). Acute stress triggers increases in blood lipids (e.g., Wirtz et al., 2009), but the long-term clinical relevance of these short-term changes, particularly for individuals with elevated stress reactivity, has not yet been fully elucidated. In addition, evidence suggests that

the basal HPA-axis activity could be an intermediate pathway for the effects of stress on cardiovascular health (Chandola et al., 2008; Knight et al., 2021). Accordingly, the dysregulation of the diurnal HPA-axis activity may affect health in multiple ways (Adam et al., 2017; Knight et al., 2021; Lupien et al., 2009; Matthews et al., 2006; O'Connor et al., 2021).

High-risk individuals, such as hypertensives and CHD-patients, are particularly vulnerable to the negative effects of stress (Kivimäki & Steptoe, 2018). Their stress system regulation seems to differ from the regulation in healthy individuals, both in response to acute stress (Walther & Wirtz, 2023; Wirtz et al., 2009) and at a basal level (Vreeburg et al., 2009; Wirtz et al., 2007). In fact, hypertension has been associated with increased blood lipid stress reactivity (Wirtz et al., 2009), and both hypertension and CHD have been related to an altered basal HPA-axis activity (Vreeburg et al., 2009; Wirtz et al., 2007). However, evidence regarding the clinical significance of these alterations particularly with respect to underlying biological mechanisms is scarce.

For this reason, the *third study* aimed to investigate potential differences in the (hemoconcentration-corrected) blood lipid stress reactivity induced using the MIST between healthy normotensive and essential hypertensive men. In a second step, we investigated whether the magnitude of the blood lipid stress reactivity would affect CHD risk in the long term. Prospective CHD risk was determined in terms of longitudinal changes in intermediate biological risk factors over a mean follow-up period of 3 years.

The *fourth study* focused on potential differences in the basal HPA-axis activity in healthy normotensive and essential hypertensive men as well as male CHD-patients. To evaluate the clinical relevance of the diurnal cortisol rhythm, we conducted further prospective investigations to determine its potential effects on changes in intermediate biological risk factors after approximately 3 years. In both studies, assessed intermediate biological CHD risk factors included markers of coagulation, inflammation, and dyslipidemia.

Empirical Study 1

Acute Stress Improves Concentration Performance: Opposite Effects of Anxiety and Cortisol

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[1] In order to ensure consistency throughout the entire text of the thesis, slight changes have been made in the following chapters covering the studies compared to the published articles (e.g., spelling of certain terms, presentation of the results and references according to the guidelines of the American Psychological Association). Therefore, the following chapters, in which the studies of the thesis are presented, may not completely replicate the published versions and are not suitable for citation.

Abstract

Acute stress can have both detrimental and beneficial effects on cognitive processing, but effects on concentration performance remain unclear. Here, we investigate the effects of acute psychosocial stress on concentration performance and possible underlying physiological and psychological mechanisms.

The study sample comprised 47 healthy male participants who were randomly assigned either to a psychosocial stress situation (Trier Social Stress Test) or a neutral control task. Concentration performance was assessed using the d2 Test of Attention before and 30 min after the stress or control task. Salivary cortisol and alpha-amylase were repeatedly measured before and up to 1 hr after stress. We repeatedly assessed state anxiety using the State-Trait Anxiety Inventory and anticipatory cognitive stress appraisal using the Primary Appraisal Secondary Appraisal questionnaire.

The stress group showed a significantly stronger improvement of concentration performance compared to the control group (p = .042). Concentration performance improvement was predicted by increased state anxiety (p = .020) and lower cortisol (stress) changes (p = .043). Neither changes in alpha-amylase nor cognitive stress appraisal did relate to concentration performance.

Our results show improved concentration performance after acute psychosocial stress induction that was predicted by higher state anxiety increases and lower cortisol increases. This points to a potential modulating role of specific psycho-emotional and physiological factors with opposite effects.

Keywords: concentration performance, cortisol, alpha-amylase, state anxiety, cognitive stress appraisal, d2 Test of Attention, Trier Social Stress Test

Introduction

The ability to concentrate or to control attention after stressful experiences, is especially crucial in professions such as surgeons or airport security personnel where small mistakes can have fatal consequences. Attention comprises multiple active cognitive processes employed to search, shift, focus, and maintain attention, and different types of attention include selective, focused, or sustained attention (Bates & Lemay, 2004). A core feature of attention is sustained attention (SA, often referred to as concentration), proposed to relate to both perception and processing of information (Krumm et al., 2008). SA, or concentration, respectively, is defined as an attentional process involving the ability to focus on a specific task while ignoring distractions (Murphy & Moran, 2012). SA enables the maintenance of vigilance, selective and focused attention, response persistence, and continuous effort, regardless of changing conditions (Cohen, 2011). In this regard, SA constitutes a prerequisite for higher cognitive and task performances in everyday and professional life (Blotenberg & Schmidt-Atzert, 2019; Krumm et al., 2008). SA assessment typically requires to constantly perform a simple mental operation in a limited time interval (Krumm et al., 2008), as in the Revision Test (Marschner, 1980) or go/no go-tasks (Scholz et al., 2009). A prominent measure of successful SA assessment in terms of concentration performance is the *d2 Test of Attention* where participants are required to cancel targets in the context of multiple distractors (Bates & Lemay, 2004; Brickenkamp, 1994).

Whereas many studies document both positive and negative effects of acute stress on cognitive processing in general (Sandi, 2013; Shields et al., 2016) with different stressors influencing cognitive processes differently (Shields et al., 2019), comparably little is known regarding the effects of acute stress on attention. So far, first studies point to differential effects of acute stress on attention with most studies in support of negative effects. Following *acute non-psychosocial stress induction* by means of aversive movie clips or examination stress

selective attention as measured by a Stroop-like task and a telephone search task (as a part of the Test of Everyday Attention) was found to be impaired as compared to the respective control condition (i.e., neutral movie clips or nonexam period; Kohn et al., 2017; Vedhara et al., 2000). Similar findings were observed following *acute psychosocial stress induction*. Participants stressed by a socially evaluated cold pressor task as compared to controls showed higher error rates and concentrated more on irrelevant stimuli in a detection task (Sänger et al., 2014) but did not differ in SA as measured in a go/no-go task (Banks et al., 2014). After acute psychosocial stress induction by the highly potent *Trier Social Stress Test* (TSST; Dickerson & Kemeny, 2004; Kirschbaum et al., 1993), SA assessed in terms of reaction times in go/no go-tasks (Scholz et al., 2009) and a card choice task (Olver et al., 2015) was found to be impaired after stress as compared to a control group or baseline, respectively. The hitherto-only study assessing SA by means of concentration performance using the d2 Test of Attention could not observe differences after a group-TSST as compared to a non-stress control condition (von Dawans et al., 2012). So far, one study found positive effects of acute stress on attention. In that study, participants were exposed to a cold pressor test and compared with a control group. The stress group responded faster in a selective attention task (flanker task) without influencing accuracy or interference control, notably without prestress baseline assessment (Shields et al., 2019). Notably, all studies assessing attention after acute psychosocial stress induction compared the stressed group either with a non-stress control group or with the baseline of the stressed group. To the best of our knowledge, published peer-reviewed studies are lacking that include both, repeated assessment of SA before and after stress, in addition to a non-stress control group.

With respect to *mechanisms* underlying the effects of acute (psychosocial) stress on attention, it has been proposed that stress may influence cognitive abilities in an inverted-U shaped function depending on perceived intensity of the stressor, with relatively moderate

physiological stress parameters relating to highest performance at or near the peak of the inverted-U (Arnsten, 2009; Domes et al., 2005). The following physiological and psychological parameters may play a role. First, stress-induced activation of the *hypothalamic-pituitary-adrenal (HPA-) axis* comprise release of circulating glucocorticoids (GCs) from the adrenal cortex (de Kloet et al., 2005) that relate to either impairment of cognitive performance or attention in a dose-dependent (Hsu et al., 2003) and potentially inverted-U shaped manner (Domes et al., 2005) or are without effect on attention including SA (Olver et al., 2015; Shields et al., 2019). Second, stress activates the *sympathetic nervous system (SNS)* with catecholamine release (de Kloet et al., 2005). Noradrenergic projections have been proposed to influence PFC function in response to stress and consequently higher-order PFC abilities such as working memory and attention regulation (Arnsten, 2009). Also, (nor)adrenergic activity is required for modulation of working memory by GCs (Elzinga & Roelofs, 2005). However, salivary alpha-amylase, a surrogate marker of stress-induced SNS activation (Kuebler, von Känel, et al., 2014; Nater & Rohleder, 2009), was not found to relate to immediate post-stress performances in attention including SA (Banks et al., 2014; Sänger et al., 2014).

With respect to psychological stress-responsive parameters, *anxiety* may play a role in mediating stress effects on SA as it can impair cognitive performance, especially when the task is complex and attentionally demanding (Derakshan & Eysenck, 2009). According to the attentional control theory, anxiety increases attention to threat-related stimuli at the expense of attentional control (Eysenck et al., 2007). Nevertheless, anxiety does not necessarily impair performance effectiveness in terms of quality as it can induce compensatory strategies such as, e.g., enhanced effort (Eysenck et al., 2007). However, to the best of our knowledge, effects of stress-induced state anxiety changes on attention or concentration performance, respectively, have not yet been investigated. *Anticipatory cognitive stress appraisal* might be a further potential modulating factor in the relationship between stress and cognitive performance, or

SA, respectively. According to the transactional model of stress, cognitive stress appraisal results from the difference between primary (comprising perceived threat and/or challenge of a stressful stimulus) and secondary appraisal (perception of the own coping abilities; Folkman et al., 1986). Indeed, one pioneer study, notably in women only, found first evidence that cognitive stress appraisal negatively relates to SA in a working memory task (digit span task; Zandara et al., 2016).

Given the above-described reasoning, we set out to investigate whether acute psychosocial stress induced by TSST as compared to a non-stress control task would relate to changes in concentration performance as measured by the d2 Test of Attention assessed repeatedly before and after the respective task. To shed light into potential underlying mechanisms, we assessed before and repeatedly after each task salivary cortisol and alpha-amylase to exploratively test for possible physiological modulation of stress effects on attention. In addition, we assessed anticipatory cognitive stress appraisal and state anxiety increases from baseline to post-task. We hypothesized that higher cortisol responses as well as higher cognitive stress appraisal and anxiety increases would predict changes in concentration performance from baseline to post-task.

Materials and Methods

Participants

The current investigation is part of a larger research project investigating the effects of acute stress on cognitive function (Thomas et al., 2014). We recruited medication-free physically and mentally healthy male volunteers aged between 18 to 40 years by online advertisement and mailing lists at the University of Zurich. Subjects were required to have normal or corrected-to-normal vision. Interested individuals were screened by a telephone interview using an extensive health questionnaire (Wirtz, Ehlert, et al., 2006). Specific

exclusion criteria as obtained by participants' self-report were the following: clinical psychosomatic and psychiatric disorders, current infectious diseases, allergies and atopic diathesis, rheumatic diseases, chronic obstructive pulmonary disease, liver and renal diseases, HIV, cancer, elevated blood sugar and diabetes, elevated cholesterol, heart disease, hypertension, varicosis, thrombotic diseases, alcohol and illicit drug abuse, smoking more than four cigarettes per day, and previous participation in studies using stress induction by TSST (Thomas et al., 2014). The initially recruited total sample size was 48 participants (Thomas et al., 2014). For this part of the study, one participant (of the control condition) had to be excluded due to incompletion of the d2 Test of Attention (please see section "Concentration Performance") after stress as main variable of interest rendering a final sample size of 47.

All study participants signed written informed consent and received research participation credits or a monetary reward of 40 Swiss Francs. The research project was approved by the institutional review board of the University of Zurich.

Design and Procedure

In anticipation of the experimental session, subjects were informed to refrain from meals and beverages other than water 1 hr prior to their appointment. Furthermore, they had to abstain from drinks containing caffeine or alcohol since the previous evening and from excessive exercise within 48 hr before study participation. In order to control for diurnal variations in cortisol secretion (Pruessner et al., 1997), experimental sessions started between 1 p.m. and 2:30 p.m. Participants were randomly assigned to either a stress (n = 24) or a control condition (n = 23). Included participants were provided with complete written and oral descriptions of the study, and all subjects expected a challenging task in the study procedure. Upon arrival, participants were welcomed and seated in a quiet room. After 15 min of resting, subjects rated their state anxiety (*State-Trait Anxiety Inventory,* STAI; see below), followed by

the baseline assessment of their concentration performance using the d2 Test of Attention (see below). One hour later, half of the participants were exposed to acute psychosocial stress (TSST; see below) in a separate room, while the others accomplished a non-stressful control task of identical duration (calm reading of newspapers and magazines). Five minutes after beginning of each task (i.e., after the introduction in the TSST group), all participants rated again their state anxiety and completed the *Primary Appraisal Secondary Appraisal* questionnaire (PASA; see below). Thirty minutes after completion of the stress or the control task, respectively, concentration performance was measured again. We decided for the 30-min interval between stress cessation and repeated concentration performance assessment as this interval is short enough to capture rapid-acting non-genomic effects of cortisol on cognitive performance and attention (Henckens et al., 2012; Shields et al., 2015). At the same time, it is long enough to allow catecholamine levels to fully recover (Nater et al., 2006) and thus eliminate or at least reduce potential catecholamine effects on concentration performance assessment. Two saliva samples for measuring salivary cortisol and alpha-amylase were taken at baseline, i.e., prior to the d2 Test of Attention, and 1 min prior to beginning of the stress or non-stress condition. Saliva samples for post-stress/non-stress cortisol and alpha-amylase assessment were taken immediately (+1 min), and 10, 20, 30, 40, 50 and 60 min after stress or non-stress cessation.

Induction of Acute Psychosocial Stress

The TSST is a well-standardized procedure to reliably induce psychosocial stress and resulting neuroendocrine responses (Dickerson & Kemeny, 2004; Kirschbaum et al., 1993). The procedure comprises three 5-min parts in a separate room in front of an unknown panel of two evaluators and a conspicuous video camera and microphone: a preparation period after a short introduction by the experimenter, a simulated job interview, and an arithmetic task. The

panel members were dressed in white laboratory coats and were presented as experts in evaluation of non-verbal behaviour. Subjects assigned to the control condition were instructed to read newspapers and magazines in a quiet room corresponding to the duration of the TSST.

Concentration Performance

To assess concentration performance before and after stress/non-stress participants completed the paper-pencil version of the d2 Test of Attention (Brickenkamp, 1994). The aim of this test is to discriminate target stimuli from visually similar distractors by cancelling letters. The test comprises 14 lines, each containing 47 randomly mixed letters. The letters are either “d” or “p” with one, two, three or four small dashes. Participants were instructed to cross out all “d” with two dashes (below and/or above) as fast and accurately as possible. They had 20 s to finish each line before the experimenter requested them to start with the next line. The test lasts for 4.40 min. Concentration performance was computed by the number of correctly canceled targets minus the number of incorrectly canceled distractors (Bates & Lemay, 2004). Reflecting speed as well as accuracy the value can be seen as an overall performance measure of the d2 Test of Attention. The d2 test has shown adequate reliability and good test-retest reliability (Brickenkamp, 2002).

Physiological Measurements

Stimulated saliva samples (by chewing on cotton roles for 1 min) for cortisol and alpha-amylase determination were collected using salivette collection devices (Sarstedt, Rommelsdorf, Germany) and stored at −20 °C until biochemical analysis. To prepare for biochemical analysis, saliva samples were thawed and centrifuged at 3,000 rpm for 10 min to yield low viscosity saliva. Salivary free cortisol concentrations were measured using a commercially available chemiluminescence immunoassay with a high sensitivity of 0.16 ng/ml (LIA; IBL Hamburg, Germany). Inter- and intra-assay variability was below 11.5% and 7.7%,

respectively. Alpha-amylase activity was determined using a commercially available enzymatic colorimetric assay according to IFCC with a lower detection limit of 3U/L (Roche diagnostics GmbH, Mannheim, Germany). Inter- and intra-assay coefficients of variance were < 10%.

Psychological Measurements

State Anxiety. To assess state anxiety, subjects completed the state scale of the German version of the STAI (Laux et al., 1981). The STAI state scale measures current anxiety as a transitory emotional state consisting of feelings of strain, worries, and nervousness in face of threatening demands. The scale comprises 20 items with responses on a 4-point Likert scale (ranging from 1 = *not at all* to 4 = *very much so*) rendering possible scores ranging from 20 to 80. The instrument asks participants to rate how they feel "right now, that is, at this moment." Sample items include "I feel calm" and "I feel nervous" with positive items being reverse coded so that higher overall scores indicate greater state anxiety. The scale has good psychometric properties with an internal consistency of $\alpha = .90$ (Laux et al., 1981).

Cognitive Stress Appraisal. Based on the transactional stress model proposed by Lazarus and Folkman (1984), anticipatory cognitive stress appraisal processes were measured by self-report using the PASA questionnaire (Gaab et al., 2005; Lazarus & Folkman, 1984). This questionnaire comprises the scales primary appraisal and secondary appraisal, each comprising eight items rated on a 6-point Likert scale (ranging from 1 = *strongly disagree* to 6 = *strongly agree*), as well as the global PASA scale, termed stress index. The global stress index scale provides an integrated measure of transactional stress perception by subtracting secondary appraisal from primary appraisal with a possible score range of −20 to 20. Higher scores in the stress index indicate higher stress appraisal. The questionnaire has good psychometric properties, showing good internal consistencies ($\alpha = .74–.80$; Gaab, 2009).

Statistical Analysis

To detect an expected effect size of .35, the sample size of $n = 48$ was calculated a priori for the previous study (Thomas et al., 2014). Statistical analysis was performed using SPSS (version 25) statistical software package for Macintosh (IBM SPSS Statistics, Somers, NY). All tests were two-tailed with the significance level set at $p < .05$. All data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests prior to statistical analyses. No outliers were excluded.

Changes in both concentration performance and state anxiety after the respective treatment were calculated as percentage changes from baseline (defined as 100%) to post-treatment. Cortisol and alpha-amylase baseline levels were calculated as the mean of the two baseline measurements. Cortisol change scores were computed by cortisol (stress) peak 10 min after the respective treatment minus baseline, and alpha-amylase change scores by peak at 1 min after the respective treatment minus baseline. All measures showing a skewed distribution (age, cortisol baseline, cortisol change, alpha-amylase change, state anxiety baseline, and concentration performance baseline and post-task levels) were log-transformed. While log-transformed data were used for modeling and testing, we depict untransformed data in Table 1.1 and in Figure 1.1 for reasons of clarity.

To calculate differences in group characteristics, we used univariate analyses of variance (ANOVAs). In order to test whether the TSST as compared to the control condition induced significant physiological stress responses, we calculated repeated measures ANOVAs with repeated assessment of cortisol and alpha-amylase, respectively (each eight repetitions, from baseline to +60 min) as dependent variable and group as independent variable.

To test our main hypothesis, whether acute stress induces changes in concentration performance that relate to physiological and/or psychological stress reactivity, we used a stepwise procedure. First, to test whether stress as compared to non-stress induces higher

increases in concentration performance, we calculated repeated measures analysis of covariance (ANCOVA) with concentration performance percentage scores as the repeated dependent variable and group as the independent variable. We controlled for baseline concentration performance and baseline cortisol levels as covariates to account for the observed significant group differences in both parameters. Notably, baseline-adjusted change-score models have been proposed to provide unbiased effect estimates (Glymour et al., 2005). Post hoc tests comprise separate reanalysis in each group. Second, to test whether concentration performance changes relate to physiological and/or psychological stress reactivity, we calculated multiple linear regression analyses with concentration performance percentage change as the dependent variable. As independent predictor variables, we entered cortisol and alpha-amylase change scores as well as state anxiety percentage change and cognitive stress appraisal (stress index) simultaneously. Huynh-Feldt correction for repeated measures was applied where appropriate.

Results

Group Characteristics

Table 1.1 provides participants' characteristics of the stress group (n = 24) and the non-stress group (n = 23). Univariate ANOVAs revealed that at baseline the two groups did not significantly differ in age and state anxiety (ps > .77). However, the stress group displayed at baseline higher concentration performance ($F(1, 45) = 6.18$, $p = .017$, partial $\eta^2 = .12$, $f = 0.37$), lower cortisol concentrations ($F(1, 45) = 4.65$, $p = .037$, partial $\eta^2 = .09$, $f = 0.31$), and a trend toward higher alpha-amylase levels ($F(1, 45) = 3.57$, $p = .065$, partial $\eta^2 = .07$, $f = 0.27$). Most stress-related measures are interrelated: Higher cortisol increases correlate with higher salivary alpha-amylase ($r(45) = .49$, $p = .001$) and state anxiety increases ($r(45) = .62$, $p < .001$) as well as higher stress appraisal ($r(45) = .46$, $p = .001$). Higher state anxiety increases correlate with

higher alpha-amylase increases ($r(45) = .33$, $p = .022$) and higher stress appraisal ($r(45) = .62$, $p < .001$). There were no associations between stress appraisal and alpha-amylase increases ($r(45) = .08$, $p = .58$).

Table 1. 1: Group Characteristics and Psychological Parameters

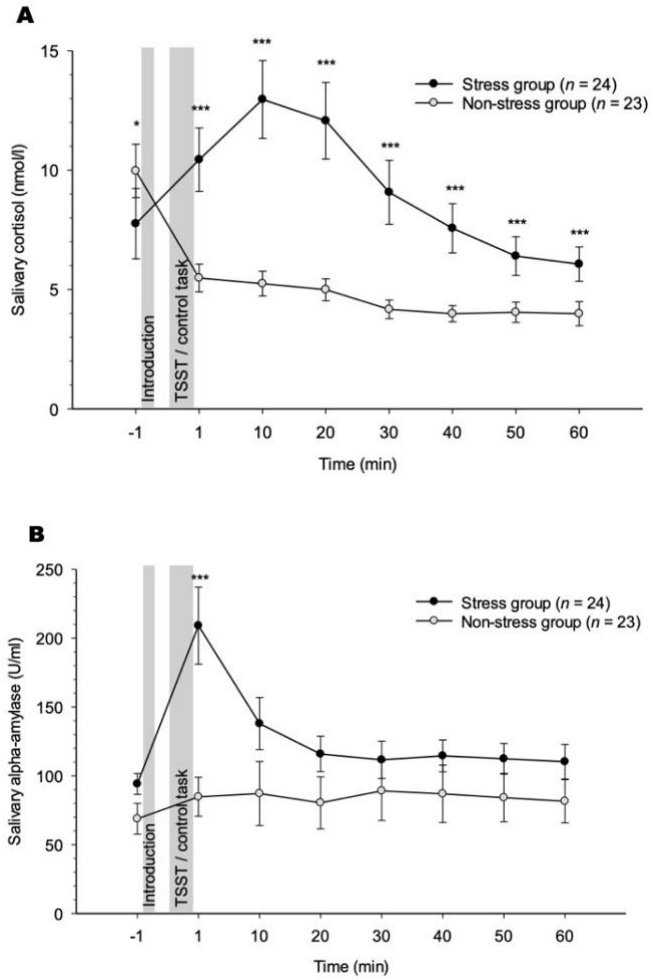
	Stress group (n = 24)	Non-stress group (n = 23)	
	M ± *SEM* (range)	*M* ± *SEM* (range)	*p*
Age, years	24.33 ± 0.89 (19–36)	24.78 ± 0.98 (19–33)	.77
Concentration performance at baseline	278.50 ± 3.33 (235–295)	263.17 ± 5.19 (206–293)	*.017*
Post-task concentration performance	287.67 ± 2.14 (261–298)	270.09 ± 5.65 (209–296)	*.006*
State anxiety at baseline	35.13 ± 1.78 (23–59)	34.83 ± 1.97 (22–57)	.82
Post-task state anxiety	46.67 ± 2.08 (27–69)	32.48 ± 1.99 (21–54)	*<.001*
Cognitive stress appraisal	−1.65 ± 1.17 (−9–11)	−7.52 ± 0.79 (−12.50–2.50)	*<.001*
Cortisol baseline levels, nmol/L	7.76 ± 1.47 (1.90–31.69)	9.97 ± 1.12 (2.86–25.21)	*.037*
Cortisol maximum change, nmol/L	5.21 ± 1.47 (−9.78–22.62)	−4.72 ± 0.69 (−12.02–0.32)	*<.001*
Alpha-amylase baseline levels, U/ml	94.17 ± 7.60 (46.05–205.05)	68.78 ± 11.20 (11.55–207.35)	.065
Alpha-amylase maximum change, U/ml	114.95 ± 25.40 (−12.05–386.30)	15.98 ± 6.89 (−34.75–114.10)	*<.001*

Note. Values are given as means ± *SEM* (range); *n* = subsample size. Significant *p*-values are highlighted in italics.

Reactivity of Stress-Reactive Physiological and Psychological Measures Over Time in the Stress and Non-stress Groups

Physiological Reactivity. As depicted in Figure 1.1, the stress group displayed in reaction to the TSST significant increases in salivary free cortisol ($F(3.3, 149.10) = 23.94$, $p < .001$, partial $\eta^2 = .35$, $f = 0.73$; Figure 1.1A) and alpha-amylase ($F(4.6, 206.8) = 3.17$, $p = .011$, partial $\eta^2 = .07$, $f = 0.27$; Figure 1.1B) as compared to the control group indicating the successful stress induction by TSST. Accordingly, cortisol ($F(1, 45) = 37.57$, $p < .001$, partial $\eta^2 = .46$, $f = 0.92$) and alpha-amylase change scores ($F(1, 45) = 16.07$, $p < .001$, partial $\eta^2 = .26$, $f = 0.59$) were higher in the stress group as compared to the control group (see Table 1.1).

Figure 1. 1: Physiological Responses to TSST and Control Task



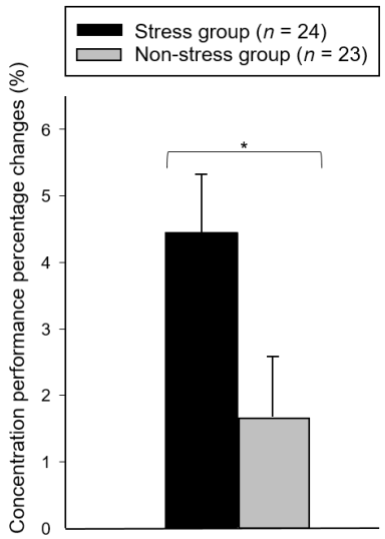
Note. Physiological responses to TSST and control task (mean ± *SEM*): (A) Salivary cortisol and (B) salivary alpha-amylase. Asterisks indicate significant group differences between stress/non-stress with post-task group differences controlled for the respective baseline levels (*$p < .05$; **$p < .01$; ***$p < .001$). TSST = Trier Social Stress Test.

Psychological Reactivity. Physiological stress reactivity results were complemented by psychological stress indicators: State anxiety significantly increased from baseline to post-treatment in the stress group as compared to the control group: $F(1, 45) = 36.84$, $p < .001$, partial $\eta^2 = .45$, $f = 0.90$). Moreover, cognitive stress appraisal in anticipation of the stress condition was significantly higher as compared to anticipation of the resting condition ($F(1, 45) = 17.00$, $p < .001$, partial $\eta^2 = .27$, $f = 0.61$; see Table 1.1).

Concentration Performance Changes in the Stress and Non-Stress Groups

Figure 1.2 depicts percentage changes in concentration performance (from baseline to post-task) in the stress and non-stress groups. Repeated measures ANCOVA revealed higher increases in concentration performance from baseline to post-task in the stress group as compared to the non-stress group independent of baseline cortisol and concentration performance ($F(1, 43) = 4.38$, $p = .042$, partial $\eta^2 = .09$, $f = 0.31$). Post hoc tests reveal that increases in concentration performance over time were significant in the stress group ($F(1, 21) = 40.35$, $p < .001$, partial $\eta^2 = .66$, $f = 1.39$), but not in the control group ($F(1, 20) = 0.16$, $p = .70$).

Figure 1. 2: Concentration Performance Increases in the Stress and the Non-Stress Group



Note. Percentage changes in concentration performance in the stress and non-stress groups (mean ± *SEM*). Independent of baseline differences in cortisol and concentration performance, percentage increases in concentration performance from baseline to post-task were higher in the stress group as compared to the non-stress group ($p = .042$). Asterisks indicate significant group differences (*$p < .05$).

Stress-Related Predictors of Changes in Concentration Performance

To test whether stress/non-stress-induced changes in concentration performance relate to physiological and/or psychological mechanisms, we calculated multiple linear regression analysis (see Table 1.2) with percentage changes in concentration performance as the dependent variable. As independent variables, we entered maximum cortisol and alpha-amylase changes as potential physiological predictors in addition to cognitive stress appraisal and percentage changes in post-task state anxiety as potential psychological predictors.

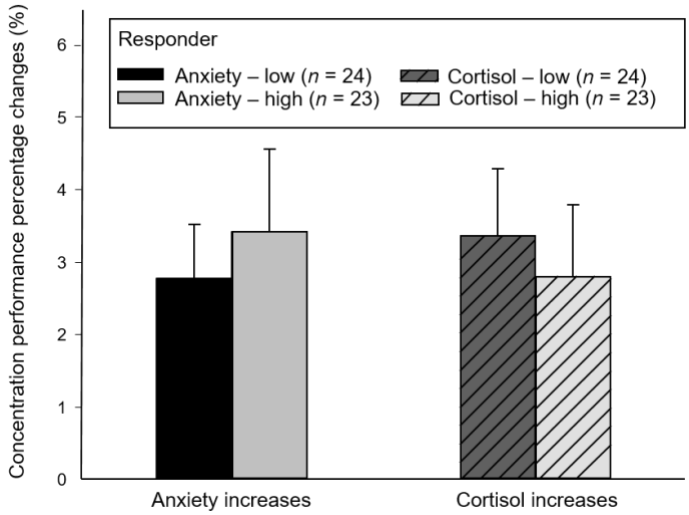
Table 1. 2: Prediction of Post-Task Percentage Changes in Concentration Performance

Variables entered	Standardized ß	*t*	*p*	ΔR^2	Tolerance	VIF
Cortisol maximum change	−.42	−2.09	*.043*	.09	.50	1.99
Alpha-amylase maximum change	.08	0.50	.62	.01	.72	1.39
State anxiety change	.51	2.42	*.020*	.12	.46	2.18
Cognitive stress appraisal	−.16	−0.86	.40	.02	.57	1.76

Note. Significant *p*-values are highlighted in in italics.

Post-task concentration performance improvement was predicted by lower cortisol increases (ß = −.42, $p = .043$, $\Delta R^2 = .09$) and higher state anxiety increases (ß = .51, $p = .020$, $\Delta R^2 = .12$). Neither task-related changes in alpha-amylase nor cognitive stress appraisal significantly related to post-task concentration performance (*p*s > .40). Tolerance and variance inflation factor (VIF) values indicate that there are no problems with multicollinearity. To graphically illustrate our findings, Figure 1.3 depicts groups with higher and lower levels of the significant stress-related predictors of concentration performance changes based on median splits.

Figure 1. 3: Anxiety and Cortisol Increases Predict Concentration Performance Increases



Note. Higher anxiety and lower cortisol increases predict higher subsequent concentration performance increases (state anxiety increases: ß = .51, $p = .020$, $\Delta R^2 = .12$; cortisol increases: ß = −.42, $p = .043$, $\Delta R^2 = .09$). The figure depicts groups with high and low anxiety increases and high and low cortisol increases based on median splits.

Discussion

The main objective of our study was to investigate whether acute psychosocial stress would relate to changes in concentration performance using the d2 Test of Attention assessed repeatedly before and after TSST or a non-stressful control task, respectively. To elucidate potential underlying physiological and psychological mechanisms, we assessed before and repeatedly after each task salivary cortisol and alpha-amylase in addition to anticipatory cognitive stress appraisal and state anxiety increases from baseline to post-task.

While most of the above-cited studies found either impairing (Olver et al., 2015; Sänger et al., 2014; Scholz et al., 2009) or no effects (Banks et al., 2014; von Dawans et al., 2012) after acute psychosocial stress in SA, our main findings indicate that acute psychosocial stress induction by means of the TSST relates to a stronger improvement in concentration performance as compared to a control task. Notably, unlike our study, these studies did either compare post-task SA assessment(s) between a stress group and a non-stress group (Banks et

al., 2014; Sänger et al., 2014; Scholz et al., 2009; von Dawans et al., 2012) or reduced SA assessment to pre-post-stress assessment in a stress group only without control group (Olver et al., 2015). Our finding of stress-induced SA improvement is in line with a previous study, where beneficial effects of cold pressor test-induced acute stress on attention performance were observed (Shields et al., 2019). The main difference between prior studies and our investigation was the use of different SA assessment methods (go/no-go task: Banks et al., 2014; Scholz et al., 2009; card choice task: Olver et al., 2015; detection task: Sänger et al., 2014; flanker task: Shields et al., 2019). Contradictory findings regarding stress effects on SA might thus result from differences in SA tasks and task difficulties. Similarly, inconsistent results of studies investigating stress effects on working memory might be explained by differences in working memory load with stress effects most apparent when working memory load is high (Oei et al., 2006). The contradictory finding of the hitherto-only study using the d2 Test of Attention who could not observe group differences between a stress and a non-stress group in SA (von Dawans et al., 2012) may relate to the timing of the d2 assessment in that study at 5 min after the end of stress induction. At this timepoint, cortisol usually starts increasing but has not yet reached peak reactivity while catecholamines are in the process of decreasing to baseline levels. Our measurement timepoint of 30 min post-stress allows to capture cortisol stress reactivity without catecholminergic influences, which may explain the differential results.

With respect to potential underlying mechanisms, we found that concentration performance improvement was predicted by lower cortisol (stress) changes and increases in state anxiety while neither changes in alpha-amylase nor cognitive stress appraisal did relate to concentration performance. Impairing cortisol effects on concentration performance improvement are in line with previous findings (Hsu et al., 2003). However, few studies could not find any relation between cortisol and attention (Olver et al., 2015; Shields et al., 2019) with potential explanations including the timing of saliva sampling omitting the cortisol stress

response (Olver et al., 2015) or the potency of the stressor to induce a strong HPA-axis activation (Shields et al., 2019). Discrepancies may relate to time dependency of GC effects with rapid, non-genomic effects followed by slower, genomic effects that may affect cognitive functions in opposite and complementary ways (Henckens et al., 2012; Shields et al., 2015). We could not find associations between concentration performance and stress-induced salivary alpha-amylase changes. On the one hand, this might be explained by our assessment time of 30 min post-stress when alpha-amylase stress reactivity has already been terminated (Nater et al., 2006). On the other hand, previous studies could not find alpha-amylase to relate to immediate post-stress performances in attention including SA (Banks et al., 2014; Sänger et al., 2014) suggesting that unlike other SNS activation markers (Arnsten, 2009), alpha-amylase may not relate to attention. Notably, as a mere surrogate marker of SNS activation, alpha-amylase, or alpha-amylase secretion, respectively, has been shown to result from catecholaminergic activation but is not causally involved in SNS activation (Kuebler, von Känel, et al., 2014; Nater & Rohleder, 2009).

With respect to anxiety, we found that stress-induced state anxiety increases related to concentration performance improvements. This finding corresponds, on the one hand, with the capacity theory that posits persons to have a greater amount of available attentional capacity when they are fully alert (Kahneman, 1973; Murphy & Moran, 2012). Notably, this reasoning is not in line with the attentional control theory where anxiety and stress impair attentional processes by forcibly narrowing attention to threat-related stimuli (Eysenck et al., 2007; Shields et al., 2019). On the other hand, persons might use compensatory strategies such as, e.g., enhanced effort in response to anxiety (Eysenck et al., 2007). Whether anxiety increases relate to stress-induced acceleration of executive motor activity (Shields et al., 2019) of relevance for letter cancelling the d2 Test of Attention remains to be elucidated. Anticipatory cognitive stress appraisal has been shown to determine cortisol stress reactivity (Gaab et al.,

2005), and in our study, stress reactivity predicted concentration performance. Indeed, previous research found cognitive stress appraisal to negatively relate to SA in a working memory task 15 min after stress cessation (Zandara et al., 2016). As we could not find any associations between cognitive stress appraisal and concentration performance 30 min after stress, we speculate that the delay between stress cessation and the second SA assessment was too long to allow anticipatory appraisal effects to persist.

Strengths of our study include that unlike previous studies we repeatedly assessed SA before and after stress in a stress group as compared to a non-stress control group. This study design allowed us to control for individual baseline differences and learning effects in concentration performance in the d2 Test of Attention. Moreover, we induced acute psychosocial stress using a well-validated and highly potent standardized laboratory stress test (Dickerson & Kemeny, 2004). A limitation of our study is that our study sample comprised healthy young Caucasian men only. Also, our study design with one single stressor of moderate intensity and two concentration performance measurement timepoints does not allow to test for quadratic and thus inverted-U shaped associations between stress-related predictors and concentration performance. Whether our findings are generalizable to populations including women, older or younger individuals, remains unclear. Despite of the random assignment of our participants to either the stress or control condition, we found baseline differences in concentration performance and salivary cortisol between the stress and non-stress group. Notably, we accounted for baseline differences in our analyses. Nevertheless, the observed baseline differences in cortisol and concentration performance between groups limit robustness of our findings. A further limitation is that as we measured SA 30 min after stress cessation when SNS stress reactivity has returned to baseline levels, our design is not well-suited to detect potential SNS effects on concentration performance. This could be one reason why we were unable to find associations between concentration performance and cognitive stress

appraisal, or salivary alpha-amylase, respectively. Also, investigation of non-invasive SNS markers other than salivary alpha-amylase would have been desirable. Moreover, the use of a placebo-TSST (Het et al., 2009) would have been a more appropriate control condition.

Our findings suggest that short-time stress or stress-induced anxiety, respectively, may have beneficial effects on concentration performance and thus SA. As we induced acute psychosocial stress using a highly potent but, compared to, e.g., life-threatening events, moderate standardized laboratory stress test, future studies are needed to elucidate the effects of acute stress of different intensities compared to our TSST. Also, effects of chronic stress or critical life events on concentration performance remain unclear. Taken together, our results suggest improved concentration performance after acute psychosocial stress induction by means of the TSST that was predicted by higher state anxiety increases and lower cortisol increases. This points to a potential modulating role of specific psycho-emotional and physiological factors with opposite effects.

Open Data: The data of all experiments reported here are accessible via PsychArchives under: http://dx.doi.org/10.23668/psycharchives.2886

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Empirical Study 2

Eating After Acute Psychosocial Stress in Healthy Men and Women: Sex Differences and Endocrine Mechanisms

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Abstract

Context. Overweight and obesity have become a major health burden with a higher prevalence of obesity in women than in men. Mental stress has been discussed to play a role in this context.

Objective. We investigated endocrine mechanisms underlying eating after acute psychosocial stress and potential sex differences therein.

Methods. A total of 32 male and 31 female healthy participants underwent the Trier Social Stress Test before they tasted ice cream in a bogus taste test 15 min after stress. We repeatedly assessed the stress hormone cortisol and the satiety hormone cholecystokinin (CCK) in saliva as well as perceived hunger before and up to 1 hr after stress.

Results. Lower immediate total cortisol stress reactivity predicted higher hunger (*p*s ≤ .004), but was not associated with food intake (*p*s ≥ .90) or total CCK release (*p*s ≥ .84). As compared to men, women ate less after stress (*p*s < .001) and had consistently lower levels of hunger (*p*s ≤ .024) and cortisol (*p*s ≤ .008) as well as a lower immediate total cortisol stress reactivity (*p*s = .002). Further, they differed in the kinetics of CCK over the total experimental procedure (*p*s ≤ .011), in immediate reaction to stress (*p*s ≤ .038), and after eating (*p*s ≤ .072), with women´s CCK levels continuously decreasing while men´s CCK levels were reactive.

Conclusion. We found evidence for lower immediate total cortisol stress reactivity relating to higher perceived hunger, with lower cortisol levels in women. Unlike men, CCK levels of women were not reactive to acute stress and eating and decreased continuously. Our results may suggest a higher risk for stress-induced eating in women.

Keywords: acute psychosocial stress, sex differences, cortisol, hunger, CCK

Introduction

The prevalence of overweight and obesity has substantially increased over the last decades, with a higher total prevalence of obesity in women as compared to men (Chooi et al., 2019). Mental stress has repeatedly been discussed to play a role in this development (e.g., Sinha & Jastreboff, 2013), but the underlying mechanisms are not clear. While most studies focused on effects of chronic stress on risk for obesity (Block et al., 2009; Brunner et al., 2007; Cotter & Kelly, 2018; Sinha & Jastreboff, 2013; Tomiyama et al., 2011; but see also Wardle et al., 2011) and eating behavior (Kim & Jang, 2017; Newman et al., 2007; Ng & Jeffery, 2003; O'Connor et al., 2008), comparably fewer studies have investigated effects of acute stress. In particular, investigation of acute stress effects on eating behavior in healthy non-obese men and women may help to understand processual aspects of underlying mechanisms.

To date, the endocrine mechanisms underlying eating behavior and related feelings of hunger in reaction to acute stress are not fully understood. At the biological level hunger and satiety are regulated by hormones of the neuroendocrine system (Ans et al., 2018; Theilade et al., 2021). In particular, appetite-stimulating hormones such as ghrelin promote food intake, while satiety-stimulating hormones such as cholecystokinin (CCK) or leptin, decrease hunger, thus promoting cessation of food consumption (Geary, 2004; Zanchi et al., 2017).

With respect to stress reactivity, ghrelin (Bouillon-Minois et al., 2021; McKay et al., 2021) and leptin (Tomiyama et al., 2012) have been shown to increase following acute mental stress induction. Similarly, CCK increases in anticipation of a sports competition (Philipp et al., 1992) but its reactivity in response to acute mental stress has not yet been investigated.

In addition to hunger and satiety hormones, the stress hormone cortisol as the end-product of a stress-induced activation of the hypothalamus-pituitary-adrenal axis may play a role in the regulation of eating after stress. A frequently used acute psychosocial laboratory stressor that induces strong physiological stress reactions, including those of cortisol, is the

Trier Social Stress Test (TSST; Dickerson & Kemeny, 2004; Kirschbaum et al., 1993). To date, a few studies compared food intake after TSST in participants with higher vs. lower cortisol responses. In normal-weight (average body mass index [BMI] < 30) women, participants with higher cortisol TSST reactivity ate more after stress as compared to those with lower cortisol TSST reactivity (Epel et al., 2001), with higher cortisol increases associated with higher food intake (Epel et al., 2001). In contrast, two other studies could not find differences (Appelhans et al., 2010; Herhaus et al., 2020) and two further studies found opposite effects with higher food intake relating to lower cortisol TSST reactivity (Klatzkin et al., 2018; Tryon et al., 2013). Results in obese participants were similarly inconclusive (Appelhans et al., 2010; Herhaus et al., 2020). In line with the studies reporting a negative association between cortisol stress reactivity and food intake (Klatzkin et al., 2018; Tryon et al., 2013), acute stress is usually accompanied by reduced feelings of appetite (Herhaus et al., 2020; Nakamura et al., 2020; Petrowski et al., 2014), hunger (van Strien et al., 2014), and desire to eat (Geliebter et al., 2012). However, studies that have investigated, notably mostly in overweight participants, the relation of hunger and appetite with stress-induced cortisol release, provide mixed findings (Geliebter et al., 2013; Herhaus et al., 2020; Petrowski et al., 2014). Finally, regarding associations between cortisol stress reactivity and hunger and satiety hormones, one study found a positive correlation between TSST-induced changes in ghrelin and cortisol in participants with and without a binge eating disorder (Rouach et al., 2007). However, leptin reactivity in response to the TSST was not correlated with cortisol in post-menopausal women (Tomiyama et al., 2012). To our best knowledge, no study to date has investigated the effects of cortisol on CCK. Taken together, studies that compare or associate cortisol stress reactivity with eating behavior, feelings of hunger, or hunger and satiety hormones have been conducted mainly in women with in part contradictory results.

Given the higher prevalence of overweight and obesity in women as compared to men, investigation of sex differences in eating behavior and feelings of hunger in reaction to acute stress may help to shed light on potential underlying mechanisms. When comparing men and women in their general eating behavior, women eat less (de Castro & Kreitzman, 1985; Rolls et al., 1991), choose healthier foods (Wardle et al., 2004; Westenhoefer, 2005), and feel more postprandial fullness than men (Monrroy et al., 2019). To the best of our knowledge only three studies investigated sex differences in eating behavior after acute mental stress, but results differed. While one study could not observe sex differences in the amount of food consumption after a mental arithmetic task (Rutters et al., 2009), men ate less after watching a stressful film compared to a control condition, whereas women tended to eat more (Grunberg & Straub, 1992). In a further study, however, men ate significantly more than women both after acute mental stress (expecting a speech task) and a control condition (Oliver et al., 2000). Sex differences in eating behavior after a potent mental stressor such as the TSST, as well as in subjective ratings of hunger, or appetite respectively, after acute stress have not yet been investigated. With respect to hunger and satiety hormone levels (for review, see Asarian & Geary, 2013) women have higher baseline levels of ghrelin, leptin (Klok et al., 2007; Williams et al., 2016) and CCK (Burton-Freeman et al., 2004; Nolan et al., 2003) compared to men. To the best of our knowledge, potential sex differences in the reactivity of hunger and satiety hormones to acute stress remain to be elucidated. In addition, studies investigating the effects of cortisol stress reactivity in eating after stress focused mainly on effects in women (Appelhans et al., 2010; Epel et al., 2001; Geliebter et al., 2013; Geliebter et al., 2012; Klatzkin et al., 2018; Tryon et al., 2013; but see also Herhaus et al., 2020; Petrowski et al., 2014) and potential sex differences still have to be clarified.

Here, we set out to investigate endocrine mechanisms underlying hunger and eating after acute psychosocial stress in healthy men and women. As endocrine measures, we

repeatedly assessed cortisol and CCK from saliva measured before and up to 60 min after stress induction. First, we investigated whether cortisol release in response to acute stress relates to food intake in a subsequent bogus taste test (BTT) starting at immediate cortisol stress reactivity 15 min after TSST (e.g., Goodman et al., 2017; Kirschbaum et al., 1993), as well as to the reactivity of CCK and sensation of hunger. To avoid sex differences in the salivary cortisol stress reactivity, we invited female participants without oral contraceptive use in the luteal phase of their menstrual cycle, as comparable cortisol reactivity in response to the TSST for men and women has been found only under these conditions (Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005). In line with the previously described reasoning, we hypothesized lower immediate (i.e., up to 15 min after stress cessation) total cortisol stress reactivity to predict higher food intake (Klatzkin et al., 2018; Tryon et al., 2013), higher hunger in reaction to stress, and correspondingly lower levels of CCK. Second, we tested for potential sex differences in CCK and hunger in immediate reaction to stress induction and quantity of food intake in the subsequent BTT. Here, we expected men to eat more than women in the taste test after stress as we considered the general sex differences in the amount of food intake (Rolls et al., 1991) to outweigh potential additional differential stress effects on food intake (Grunberg & Straub, 1992). With respect to feelings of hunger, we hypothesized men to repeatedly report higher levels of hunger throughout the experiment. Moreover, given the baseline differences in satiety hormones (Burton-Freeman et al., 2004; Klok et al., 2007; Nolan et al., 2003; Williams et al., 2016) and the food intake differences after stress between men and women (Grunberg & Straub, 1992), we expected sex differences in CCK in immediate reaction to stress and after food intake.

Materials and Methods

Study Participants

We recruited apparently healthy male and female volunteers aged between 18 and 48 years by advertisement and mailing lists. Interested individuals were screened by an online questionnaire asking for the following exclusion criteria: excessive alcohol or drug use, smoking more than five cigarettes per day, acute as well as chronic physical or mental illness, medication, use of oral contraceptives, and food intolerances. To compare immediate cortisol stress reactivity between men and women, eligible female participants were invited during the luteal phase of their menstrual cycle based on information regarding the date of the 1st day of their last menstruation and the typical length of their menstrual cycle (Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005). All participants provided written informed consent and were compensated with €20 or three subject hours. The study was approved by the ethics committee of the University of Konstanz.

Design and Procedure

In anticipation of the experimental session, participants were asked to refrain from coffee on the study day. In addition, they had to abstain from meals and beverages other than water and not to brush their teeth 2 hr prior to their appointment. Furthermore, they had to abstain from excessive exercise within 48 hr before study participation. To control for diurnal variations in cortisol secretion (Pruessner et al., 1997), experimental sessions started between 2 p.m. and 4 p.m. On arrival, participants were welcomed and seated in a quiet room before they were provided with complete written and oral descriptions of the study. The study participants were informed that they would be engaged in a challenging task and a taste test during the course of the experimental procedure. Participants were administered 200 ml of grape juice 40 min prior to the start of the TSST (discussed later; Zänkert et al., 2020) before

they completed questionnaires. Participants´ height and weight (Sanitas SBG39) were measured before they were exposed to the TSST in a separate room. Fifteen minutes after the stress cessation actual food intake was assessed during a 15-min BTT (Sproesser et al., 2014; discussed later). Saliva samples for cortisol and CCK assessment were taken immediately before (−1 min [S1] and 1 min [S2], and 10 [S3], 20 [S4], 30 [S5], 45 [S6], and 60 min [S7] after stress cessation. While samples S1 to S3 allow for testing of immediate (total) cortisol stress reactivity alone, the subsequent samples assess immediate reactivity to food intake during the BTT with assessment before (S3), during (S4) and after food intake (S5–S7). In addition, the sensation of hunger (using a visual analog scale) was recorded at each sampling timepoint before and after stress.

Induction of Acute Psychosocial Stress

The TSST is a well-standardized procedure to reliably induce psychosocial stress and resulting neuroendocrine responses (Dickerson & Kemeny, 2004; Kirschbaum et al., 1993). The procedure comprises a preparation period after a short introduction by the experimenter (5 min), a simulated job interview (5 min), and an arithmetic task (5 min). The test took place in a separate room in front of an unknown panel of two evaluators and a conspicuous video camera and microphone. The panel members, dressed in white laboratory coats, were introduced as experts in evaluation of non-verbal behavior.

Bogus Taste Test

BTTs are used to assess actual food intake and simultaneously omit potential biases of self-reports and retrospective memories of eating behavior (Sproesser et al., 2014). Fifteen minutes after stress cessation, three different flavors of ice cream (each approximately 72 g and 225.3 kcal) were served to the participants, who were asked to evaluate the taste, texture, and their preference of the flavors. In detail, the taste test included 20 fake questions per ice cream

flavor (e.g., “How much do you like this ice cream?” or “How likely is it that you would purchase this ice cream?”) to be answered on a 4-point scale (Sproesser et al., 2014). Participants were told to taste and eat as much as they liked in the following 15 min. As outcome, ice cream bowls were weighed before and after the taste test to calculate the quantity of ice cream (in grams) that had been consumed.

Repeated Assessment of Hunger

To assess sensations of hunger before and after stress as well as before and after the BTT, that is, food intake, participants evaluated their current hunger ("Do you feel hungry right now?") and on a visual analog scale ranging from 0 (“Not hungry at all”) to 10 (“Very hungry”) with each salivette at each sampling timepoint (i.e., −1, 1, 10, 20, 30, 45, and 60 min ([S1–S7]).

Biochemical Analyses

Saliva samples for cortisol and CCK determination were collected using salivettes (Sarstedt), centrifuged and aliquoted into Eppendorf tubes, and stored at −20 °C until biochemical analyses. To prepare biochemical analyses, saliva samples were thawed and centrifuged at 2500g for 10 min. Biochemical analyses of cortisol were performed using a competitive enzyme-linked immunosorbent assay (ELISA, IBL International GmbH; Tecan [IBL] catalog No. RE52611, RRID:AB_3064818). Intra-assay and inter-assay coefficients of variation were ≤ 13.2%. CCK was determined using a competitive inhibition enzyme immunoassay for the in vitro quantitative measurement of CCK (ELISA, Biomatik Corporation; Biomatik catalog No. EKL54157, RRID:AB_3064819). Intra- assay and inter-assay coefficients of variation were ≤ 12%. Due to insufficient amount of saliva, CCK could not be determined sufficiently (missing baseline and at least two further missing timepoints)

analyzed in four male participants. These four men were excluded from analyses including CCK measures.

Statistical Analyses

Statistical analyses were performed using SPSS (version 26.0) statistical software packages for MacIntosh (IBM SPSS Statistics). All tests were two-tailed with level of statistical significance set at $p < .05$. No outliers were excluded. We determined *f* from partial η^2 (η^2_p) values using G*Power3.1. Effect size parameters *f* and R^2 changes are reported where appropriate (effect size conventions; small: $f = 0.10$, $\Delta R^2 = .02$; medium: $f = 0.25$, $\Delta R^2 = .13$; large: $f = 0.40$, $\Delta R^2 = .26$; Cohen, 1988). For all participants, we calculated BMI by the formula BMI = kg/m^2.

To compute sex differences in participant characteristics as well as baseline assessments of cortisol and eating-related parameters (i.e., CCK, hunger and, quantity of food intake) we used univariate analysis of variance (ANOVA). To confirm that the TSST significantly induced immediate cortisol stress reactivity, we calculated repeated-measures analysis of covariance (ANCOVA) with repeated assessment of cortisol (S1–S7) as manipulation check.

To analyze our first study question, that is, potential stress-induced cortisol effects on eating-related measures, we aggregated the immediate total cortisol stress reactivity in response to the TSST as area under the curve with respect to ground (AUC_G; Pruessner et al., 2003) from baseline to 10 min after the TSST and thus before beginning the BTT ($AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$). We first calculated a linear regression analysis with cortisol $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$ as a predictor and food intake as the dependent variable. Second, we tested for potential effects of immediate total cortisol stress reactivity on hunger and CCK levels over the course of the experimental procedure. For this purpose, we aggregated the repeated measures of hunger and CCK

respectively as AUC_Gs from baseline to 60 min after the TSST (hunger $AUC_{G\text{-}S1\text{-}S7}$; CCK $AUC_{G\text{-}S1\text{-}S7}$). We calculated regression analyses with cortisol $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$ as the independent variable and aggregated hunger ($AUC_{G\text{-}S1\text{-}S7}$) or CCK levels ($AUC_{G\text{-}S1\text{-}S7}$) as the respective dependent variable. Post hoc testing of significant regression analyses comprised separate regression analyses for the different phases of the experimental procedure, that is, stress peak reactivity alone (Peak-S1-S3), immediate reactivity to food intake after stress during the BTT (BTT-S3-S5), and the final phase after completion of food intake (After-BTT-S5-S7). We therefore computed AUC_Gs of significant repeatedly assessed dependent variables for the three phases of the experimental procedure ($AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$, $AUC_{G\text{-}BTT\text{-}S3\text{-}S5}$, $AUC_{G\text{-}After\text{-}BTT\text{-}S5\text{-}S7}$). To determine possible interactions with immediate total cortisol stress reactivity, complementary post hoc testing comprised repeated-measures ANCOVAs with significant dependent variables for the three phases of the experimental procedure (i.e., S1–S3, S3–S5, and S5–S7) and immediate total cortisol stress reactivity (cortisol $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$) as the linear independent variable.

Our second study aim was to test for potential sex differences in CCK and hunger in reaction to stress induction and quantity of food intake in the subsequent BTT. We calculated a repeated-measures AN(C)OVA with repeated assessment of cortisol (S1–S7) as dependent and sex as the independent variable to test whether cortisol stress reactivity would be comparable between men and women in the luteal phase of the menstrual cycle.

To reveal potential sex differences in the endocrine mechanisms of hunger and eating after acute psychosocial stress, we first calculated an univariate AN(C)OVA with quantity of food intake as the dependent variable and sex as the independent variable. Second, we calculated repeated-measures AN(C)OVAs with CCK and hunger (S1–S7) as dependent variables. Post hoc testing of significant repeated-measures AN(C)OVAs comprised separate analyses of CCK and hunger in the different phases of the experimental procedure.

We conducted all analyses of study question 1 with the control for potential confounding effects of sex alone in addition to age and BMI. Sex differences in study question 2 were analyzed without and with controlling for age and BMI. We aimed at recruiting women in the luteal phase of their menstrual cycle. However, post hoc verification of cycle phase revealed that on the experimental day three women were in the follicular phase. Therefore, we additionally controlled for cycle phase in all cortisol analyses. We applied Huynh-Feldt correction for repeated measures.

All data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests prior to statistical analyses. All measures showing a skewed distribution (age, BMI, repeated cortisol, CCK and hunger measures as well as cortisol $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$, cortisol $AUC_{G\text{-}BTT\text{-}S3\text{-}S5}$, cortisol $AUC_{G\text{-}After\text{-}BTT\text{-}S5\text{-}S7}$ and CCK $AUC_{G\text{-}S1\text{-}S7}$) were log-transformed. While log-transformed data were used for modeling and testing, we depict untransformed data in Table 2.1 and in Figures 2.1 to 2.5.

Results

Participant Characteristics

Table 2.1 provides the demographic characteristics and eating-related measures of the 32 male and 31 female participants. Men and women did not differ in age and BMI (*p*s ≥ .22), although men showed a higher food intake ($p < .001$) after stress as well as higher cortisol (with and without control for menstrual cycle phase: $p = .002$) and hunger baseline levels ($p = .051$). Women had higher levels of baseline CCK towards a trend level of significance ($p = .093$).

Cortisol Stress Reactivity as a Predictor of Eating-Related Measures

Cortisol. Across all participants, the TSST induced significant increases in cortisol (main effect of time: S–S7: $F(2.40, 146.46) = 4.50$, $p = .008$, $\eta^2_p = .07$, $f = 0.27$; with all

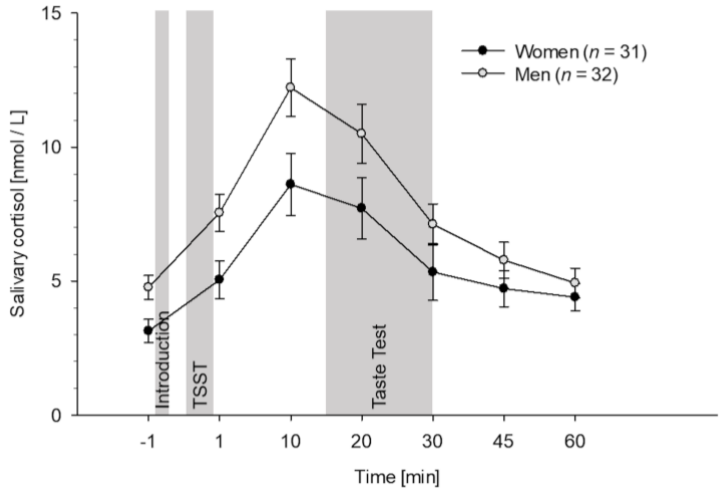
covariates: $F(2.63, 152.46) = 3.30$, $p = .027$, $\eta^2_p = .05$, $f = 0.23$), with highest levels observed 10 min after stress cessation (see Figure 2.1).

Table 2. 1: Participant Characteristics and Baseline Assessment

	N = 63	Men (*n* = 32)	Women (*n* = 31)	*p*
Age, years	23.22 ± 0.72 (18–48)	22.97 ± 0.97 (18–48)	23.48 ± 1.09 (18–46)	.74
BMI	22.79 ± 0.41 (17.89–34.58)	23.23 ± 0.54 (17.89–30.12)	22.33 ± 0.63 (17.94–34.58)	.22
Food intake, g	121.98 ± 7.31 (6.42–212.21)	147.38 ± 9.83 (56.36–212.21)	95.75 ± 8.75 (6.42–185.71)	<.001
Cortisol, nmol/L (S1)	3.96 ± 0.33 (0.63–10.71)	4.76 ± 0.45 (1.45–10.60)	3.13 ± 0.43 (0.63–10.71)	.002
$AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$	160.86 ± 11.15 (26.36–435.94)	191.17 ± 14.80 (56.82–356.11)	129.57 ± 14.99 (26.36–435.94)	.002
$AUC_{G\text{-}BTT\text{-}S3\text{-}S5}$	174.69 ± 14.63 (12.41–660.47)	201.64 ± 18.47 (49.68–476.93)	146.87 ± 22.02 (12.41–660.47)	.014
$AUC_{G\text{-}After\text{-}BTT\text{-}S5\text{-}S7}$	160.60 ± 14.17 (16.56–663.44)	176.99 ± 18.94 (39.02–583.33)	143.68 ± 21.02 (16.56–663.44)	.078
CCK, pg/ml (S1)	372.22 ± 54.79 (7.75–2071.94)	297.83 ± 63.00 (7.75–1405.91) (*n* = 28)	439.42 ± 86.62 (15.58–2071.94)	.093
Hunger (S1)	4.02 ± 0.36 (0.00–9.79)	4.70 ± 0.51 (0.00–9.79)	3.33 ± 0.49 (0.00–9.38)	.051

Note. Abbreviations: AUC_G, area under the curve with respect to ground; BMI, body mass index; BTT, bogus taste test; CCK, cholecystokinin.

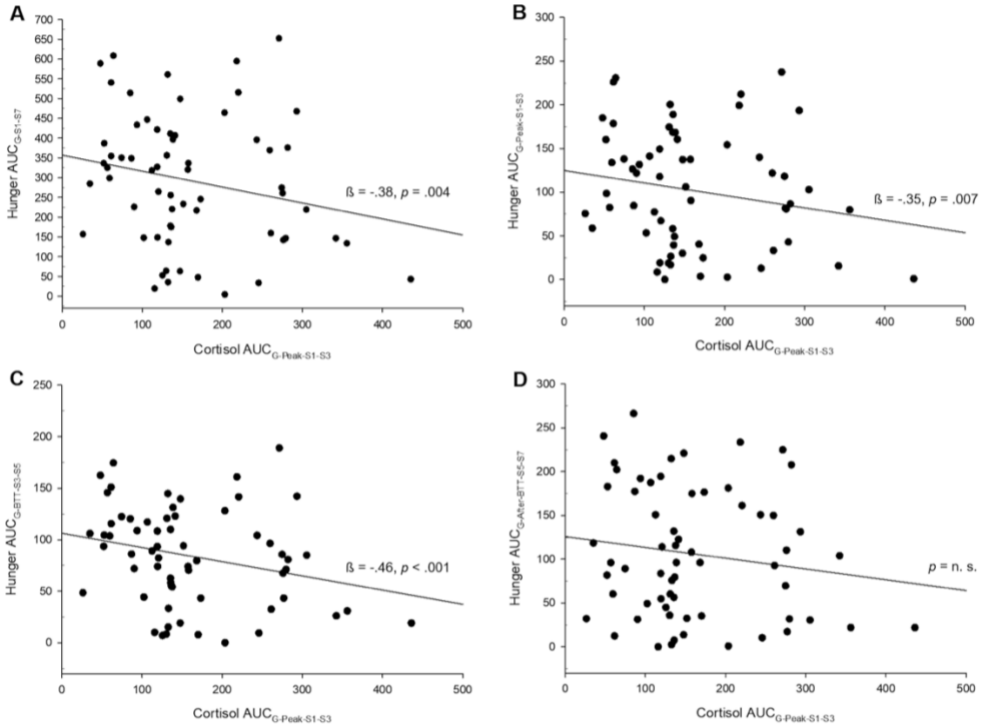
Figure 2. 1: Cortisol Stress Reactivity



Note. Cortisol reactivity of men and women over the total experimental procedure: stress peak reactivity (S1–S3), immediate reactivity to food intake after stress during the bogus taste test (S3–S5), and after the bogus taste test (S5–S7; mean ± *SEM*).

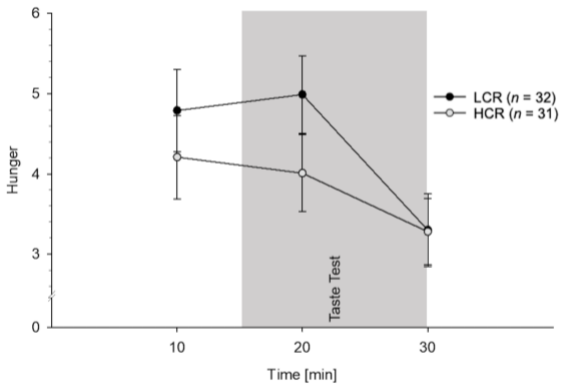
Eating-Related Parameters. Immediate total cortisol stress reactivity in terms of AUC_G from baseline to peak ($AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$) did not predict the amount of food intake (in grams) and thus eating after stress (*p*s ≥ .90) nor was it associated with CCK $AUC_{G\text{-}S1\text{-}S7}$ (*p*s ≥ .84). However, lower cortisol $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$ predicted higher hunger over the total experimental procedure (hunger $AUC_{G\text{-}S1\text{-}S7}$: ß = −.38, $p = .004$, $\Delta R^2 = .20$; with all covariates: ß = −.39, $p = .003$, $\Delta R^2 = .24$, see Figure 2.2). Post hoc analyses revealed that lower cortisol $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$ predicted higher hunger in particular during peak reactivity from S1 to S3 (hunger $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$: ß = −.35, $p = .007$, $\Delta R^2 = .19$; with all covariates: ß = −.36, $p = .007$, $\Delta R^2 = .21$) as well as in immediate reaction to food intake from S3 to S5 (hunger $AUC_{G\text{-}BTT\text{-}S3\text{-}S5}$: ß = −.46, $p < .001$, $\Delta R^2 = .27$; with all covariates: ß = −.48, $p < .001$, $\Delta R^2 = .31$). After food intake (S5–S7), associations between cortisol and hunger disappeared (*p*s ≥ .075). There was a significant interaction effect of immediate total cortisol stress reactivity with hunger in reaction to food intake (S3–S5: $F(1.69, 101.321) = 3.86$, $p = .031$, $\eta^2_p = .06$, $f = 0.25$; with all covariates: $F(1.78, 101.59) = 3.67$, $p = .034$, $\eta^2_p = .06$, $f = 0.25$; see Figure 2.3). There were no further interactions with hunger, neither in reaction to stress (S1–S3: *p*s ≥ .25) nor after food intake (S5–S7: *p*s ≥ .47).

Figure 2. 2: Effects of the Immediate Total Cortisol Stress Reactivity on Hunger During the Different Phases of the Experimental Procedure



Note. The effects of the immediate total cortisol stress reactivity ($AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$) on hunger over A, the total experimental procedure (Hunger $AUC_{G\text{-}S1\text{-}S7}$); B, during stress peak reactivity (Hunger $AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$); C, in immediate reaction to food intake after stress during the bogus taste test (Hunger $AUC_{G\text{-}BTT\text{-}S3\text{-}S5}$); and D, after the bogus taste test (Hunger $AUC_{G\text{-}After\text{-}BTT\text{-}S5\text{-}S7}$).

Figure 2. 3: Interaction Effect of the Immediate Total Cortisol Stress Reactivity and Hunger



Note. Interaction effect of the immediate total cortisol stress reactivity and hunger in reaction to food intake. The figure depicts hunger levels from S3 to S5 for individuals with immediate total cortisol stress reactivity higher (HCR) and lower (LCR) than median (mean ± *SEM*).

Sex Differences in Cholecystokinin, Hunger, and Food Intake After Stress

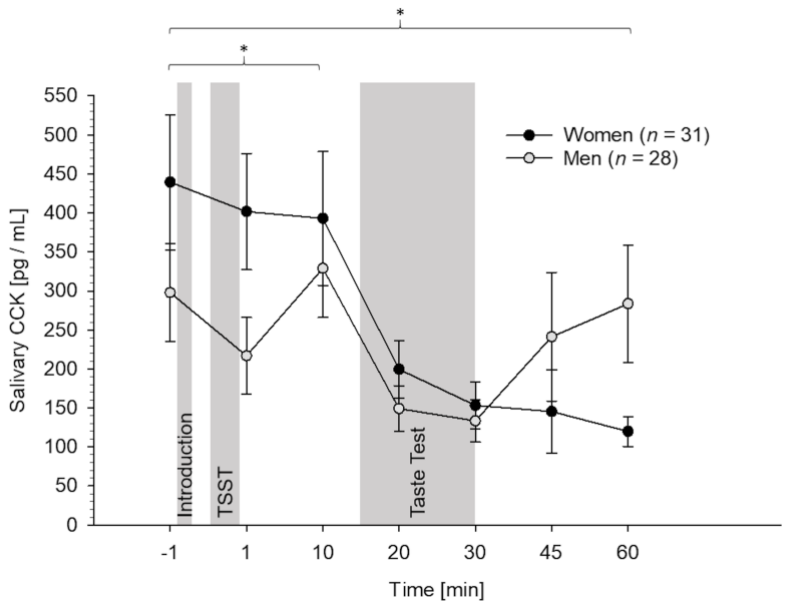
Cortisol. As intended by our recruiting procedure, men and women did not differ in their cortisol reactivity in response to the TSST (interaction sex-by-time, *p*s ≥ .10, see Figure 2.1). However, there was a main effect of sex with overall higher cortisol secretion in men (S1–S7: without covariates: $F(1, 61) = 8.56$, $p = .005$, $\eta^2_p = .12$, $f = .37$; with all covariates: $F(1,58) = 7.59$, $p = .008$, $\eta^2_p = .12$, $f = 0.37$). As compared to men, women showed lower immediate total cortisol stress reactivity ($AUC_{G\text{-}Peak\text{-}S1\text{-}S3}$: without covariates: $F(1, 61) = 10.88$, $p = .002$, $\eta^2_p = .15$, $f = 0.42$; with all covariates: $F(1, 58) = 10.36$, $p = .002$, $\eta^2_p = .15$, $f = 0.42$) and lower total cortisol reactivity to eating ($AUC_{G\text{-}BTT\text{-}S3\text{-}S5}$: without covariates: $F(1, 61) = 6.46$, $p = .014$, $\eta^2_p = .10$, $f = 0.33$; with all covariates: $F(1, 58) = 5.18$, $p = .027$, $\eta^2_p = .08$, $f = 0.29$) but they did not differ in their total cortisol after food intake ($AUC_{G\text{-}After\text{-}BTT\text{-}S5\text{-}S7}$: *p*s ≥ .078; see Table 2.1).

Eating-related Parameters. Men ate significantly more ice cream after stress than women (without covariates: $F(1, 61) = 15.33$, $p < .001$, $\eta^2_p = .20$, $f = 0.50$; with covariates: $F(1, 59) = 12.48$, $p < .001$, $\eta^2_p = .18$, $f = 0.47$). Moreover, men and women significantly differed in the kinetics of CCK secretion (interaction sex-by-time, S1–S7: without covariates: $F(4.01, 228.57) = 3.83$, $p = .005$, $\eta^2_p = .06$, $f = 0.25$; with covariates: $F(4.09, 224.94) = 3.29$, $p = .011$, $\eta^2_p = .06$, $f = 0.25$; see Figure 2.4; main effect of gender: *p*s ≥ .30). While women had borderline significantly higher CCK levels at baseline (S1: without covariates: $F(1, 57) = 2.93$, $p = .093$; with covariates; $F(1, 55) = 3.36$, $p = .072$, see Table 2.1), post hoc analyses revealed that women´s CCK levels continuously decreased after stress while men´s CCK levels showed a stress reaction with levels decreasing immediately after stress and recovering until 10 min after stress cessation (interaction sex-by-time, S1–S3: without covariates: $F(1.94, 110.56) = 3.65$, $p = .030$, $\eta^2_p = .06$, $f = 0.25$; with covariates: $F(2.0, 110.0) = 3.36$, $p = .038$, $\eta^2_p = .06$, $f = 0.25$; main effect of sex: *p*s ≥ .08). While men and women did not differ in their CCK reactivity in

immediate reaction to eating (interaction sex-by-time, S3–S5: *p*s ≥ .64; main effect of sex: *p*s ≥ .25). While CCK further decreased after eating in women, it increased in men at a trend level towards significance (interaction sex-by-time, S5–S7: without covariates: $F(1.77, 101.05) = 3.15$, $p = .053$; with covariates: $F(1.85, 101.93) = 2.76$, $p = .072$; main effect of sex, *p*s ≥ .60).

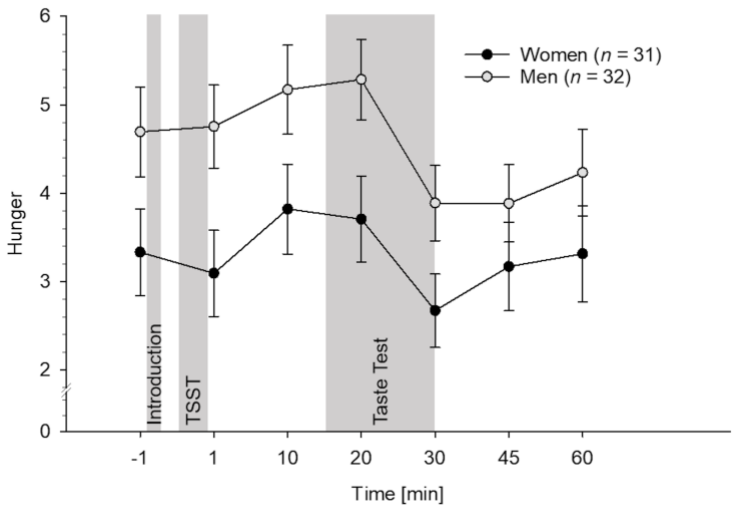
In terms of hunger, women indicated lower overall levels of hunger (main effect of sex, S1–S7: without covariates: $F(1, 61) = 6.00$, $p = .017$, $\eta^2_p = .09$, $f = 0.31$; with covariates: $F(1, 59) = 5.38$, $p = .024$, $\eta^2_p = .08$, $f = 0.29$), but men and women did not significantly differ in their reactivity over the course of the experimental procedure (interaction sex-by-time, S1–S7: *p*s = .72, see Figure 2.5).

Figure 2. 4: Cholecystokinin Secretion Over the Total Experimental Procedure



Note. Cholecystokinin (CCK) secretion of men and women over the total experimental procedure: stress peak reactivity (S1–S3), immediate reactivity to food intake after stress during the bogus taste test (S3–S5), and after the bogus taste test (S5–S7; mean ± *SEM*). Asterisks indicate significant sex differences in the kinetics of CCK secretion (*$p < .05$).

Figure 2. 5: Hunger Over the Total Experimental Procedure



Note. Hunger of men and women over the total experimental procedure: stress peak reactivity (S1–S3), immediate reactivity to food intake after stress during the bogus taste test (S3–S5), and after the bogus taste test (S5–S7; mean ± *SEM*).

Discussion

Here, we set out to investigate endocrine mechanisms underlying hunger and eating after acute psychosocial stress in healthy men and women. We repeatedly assessed the stress hormone cortisol and the satiety hormone CCK from saliva measured before and up to 60 min after stress induction in terms of the TSST. Our first study aim was to investigate whether the immediate total cortisol stress reactivity relates to food intake after stress, as well as to the reactivity of CCK and sensation of hunger in immediate reaction to stress induction, in reaction to the BTT, and after the taste test.

Our results showed for the first time that lower immediate total cortisol stress reactivity in response to the TSST significantly related to a higher perceived hunger over the entire course of the experimental procedure, and in particular, in immediate response to the TSST, in response to the taste test and thus eating, and toward a trend level of significance after food

intake. Our results point to potential endocrine underpinnings of previous study findings of reduced feelings of appetite (Herhaus et al., 2020; Nakamura et al., 2020; Petrowski et al., 2014), hunger (van Strien et al., 2014), and desire to eat (Geliebter et al., 2012) after acute stress as compared to non-stress. A reduced feeling of hunger and food intake after acute stress has been interpreted as an appropriate and adaptive reaction, allowing the body to prepare for a fight-or-flight response (e.g., Kiessl & Laessle, 2016; Torres & Nowson, 2007), which according to our findings may be regulated by the amount of the cortisol stress response. Moreover, our results of a negative association between cortisol and hunger are in line with evidence from patients with seasonal depression who have both, attenuated circadian cortisol secretion (Thorn et al., 2011) on the one hand and increased appetite and weight gain on the other hand (Praschak-Rieder & Willeit, 2003). The effects of the immediate total cortisol stress reactivity on hunger might not, however, be independent of the type of stressor, as a previous study using Cold Pressor Test and thus a physical stressor to induce stress found a positive association between cortisol stress reactivity and hunger in overweight women (Geliebter et al., 2013).

In contrast to our hypotheses and to the previously described results regarding hunger, immediate total cortisol stress reactivity was not related to the amount of food intake after stress. Most previous studies were able to demonstrate a relationship between the stress-induced cortisol response and the amount of food intake in (on average) normal-weight women (Epel et al., 2001; Klatzkin et al., 2018; Tryon et al., 2013) and obese participants (Appelhans et al., 2010; Herhaus et al., 2020), although the direction of the associations varied considerably. In line with our results, a previous study in lean participants could not find differences concerning eating behavior after acute stress in cortisol high reactors as compared to cortisol low reactors (Appelhans et al., 2010). In that study, participants were encouraged to eat by reminding them that they had not eaten since morning and by telling them that uneaten

food would be discarded (Appelhans et al., 2010). It is possible that such statements put the participants under pressure, resulting in biased food intake notably in lean participants. Although our BTT was successfully used in an earlier study in the context of stress and eating (Sproesser et al., 2014), our participants may not have felt comfortable eating as much or as little as they wanted in the time-limited taste test, possibly leading to a bias in our results.

In line with a previous TSST study (Tomiyama et al., 2012), in which reactivity of the satiety hormone leptin was unrelated to cortisol reactivity, we could not find an association between immediate total cortisol stress reactivity and CCK release, indicating that cortisol release during acute stress might not relate to satiety hormones in general. We interpret these findings in that the negative effects of the immediate total cortisol stress reactivity on hunger after acute stress seem to be mediated differently, most likely on a more central level. More precisely, food intake and energy homeostasis are regulated centrally, mainly in the arcuate nucleus of the hypothalamus (Alhabeeb et al., 2021; Huda et al., 2006; Sam et al., 2012). The arcuate nucleus comprises among others the orexigenic peptides neuropeptide Y and agouti-related peptide, which both stimulate food intake (Huda et al., 2006; Sam et al., 2012). Evidence from a rodent study suggests that stress-induced hypothalamus-pituitary-adrenal axis activation may reduce the number and expression of agouti-related peptide-producing cells and thus act as an anorexigenic substance (Ans et al., 2018; Chagra et al., 2011).

Our second study aim was to test for potential sex differences in CCK and hunger in reaction to our experimental procedure and quantity of food intake acute after stress. In line with our hypotheses and with previous studies pointing to a lower food intake in women in general (de Castro & Kreitzman, 1985; Oliver et al., 2000; Rolls et al., 1991) and after acute stress (Oliver et al., 2000), women ate significantly less ice cream during the taste test and reported a lower perception of hunger over the course of the experimental procedure than men. Regarding men´s higher body weights and consequently greater daily energy requirements

(Oliver et al., 2000), these results have not been surprising. Notably, there were no reactivity differences in hunger in reaction to the experimental procedure between men and women. Our recruiting procedure with women recruited in the luteal phase of the menstrual cycle (but with three exceptions) was intended to prevent sex differences in the kinetics of the cortisol stress reactivity. Indeed, despite a main effect of sex with women showing overall lower cortisol levels and thus a lower immediate total cortisol stress reactivity, the kinetics of the reactivity of cortisol in response to stress, that is, the interaction sex-by-time, did not differ between men and women. Given this comparable cortisol stress reactivity and given that a previous study reported higher psychological reactivity to the TSST in women compared with men (Kelly et al., 2008), we do not assume that the TSST was less stressful for women than for men. Notably, women in general (except for women without hormonal contraceptives in the luteal phase of the menstrual cycle) show lower cortisol responses to acute stress than men (Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005). This generally lower cortisol reactivity in women together with our results of lower immediate total cortisol stress reactivity (even in the luteal phase) and the observed negative association between immediate total cortisol stress reactivity and perceived hunger, may suggest that women have a lower stress-induced hunger inhibition than men. Future studies are needed to test if the lower total cortisol reactivity to stress in women induces a lower hunger inhibition as compared to non-stress, that in turn puts women at higher risk for stress-related eating.

With respect to CCK, men and women differed in the kinetics of CCK over the course of the experiment. This difference was evident at baseline, with women having slightly higher CCK levels than men (of borderline significance). This finding is in line with previous studies pointing to higher gastrointestinal hormone levels (Klok et al., 2007; Williams et al., 2016) including CCK (Burton-Freeman et al., 2004; Nolan et al., 2003) in women. The difference in CCK between men and women became more evident during the immediate response to stress,

with women´s CCK levels constantly decreasing while men´s CCK levels showed a stress reaction with levels decreasing immediately after stress and recovering until 10 min after stress cessation. In addition, we found that CCK further decreased in women after eating while it tended to increase in men. This finding differs from a previous study in which women had a greater prandial CCK secretion than men (Nolan et al., 2003). Notably, in that study there was no stress induction before eating, which may point to a potential effect of previous acute psychosocial stress induction. Taken together, our results with consistent decreases and lack of CCK reactivity, both in reaction to acute stress and to eating after stress in women, suggest that the CCK secretion seems to be less reactive in women than in men. Accordingly, women could be at higher risk for stress-induced eating, as the observed missing CCK increases, in particular in response to eating (Huda et al., 2006), could possibly lead to a lower satiation (Huda et al., 2006) and thus prevent the termination of food intake (Geary, 2004). Notably, additional controlling for the menstrual cycle phase regarding analyses of sex differences in the amount of food intake, hunger, and CCK did not substantially change results except that the main effect of sex in hunger (S1–S7: with covariates age and BMI: $F(1, 59) = 5.38$, $p = .024$, $\eta^2_p = .08$, $f = 0.29$; with covariates age, BMI, and menstrual cycle phase: $F(1, 58) = 3.01$, $p = .088$, $\eta^2_p = .05$, $f = 0.23$) became borderline significant.

Taken together, despite healthier general eating habits compared to men, i.e., by eating less (de Castro & Kreitzman, 1985; Rolls et al., 1991) and choosing healthier foods (Wardle et al., 2004; Westenhoefer, 2005), our findings on immediate total cortisol stress reactivity and related hunger combined with the satiety hormone CCK suggest that women might be at higher risk for stress-related eating after acute stress. Indeed, studies investigating the effects of chronic stress on eating behavior confirm that women are at higher risk for increased stress-related food consumption (Cotter & Kelly, 2018; Du et al., 2022; Stone & Brownell, 1994; Zellner et al., 2006) and weight gain (Serlachius et al., 2007). In this context, uncontrolled and

emotional eating in women but not in men has been proposed to play a mediating role (Du et al., 2022). However, whether our findings point to potential endocrine mechanisms underlying higher stress-related obesity risk in women remains to be elucidated. With respect to clinical implications, light therapy has been shown to elevate low cortisol levels in the morning (Leproult et al., 2001) and to reduce body fat and appetite in overweight women (Danilenko et al., 2013). Future studies are needed to investigate the effects of light therapy in the context of stress-related eating.

Strengths of our study include the fact that our study sample comprised men and women, allowing us to test for sex differences in eating after acute stress. Further, we invited female participants in the luteal phase of their menstrual cycle to allow for comparable cortisol stress reactivity between men and women (Kajantie & Phillips, 2006; Kirschbaum et al., 1999; Kudielka & Kirschbaum, 2005).

A limitation of our study is the use of a cover story (i.e., the BTT) to encourage the participants to eat after stress induction, which may have led to a bias in our results. Our study design without a non-stress control condition was not intended and consequently did not allow to investigate direct stress effects on the amount food intake as well as hunger or CCK kinetics as compared to non-stress. Therefore, investigation of direct stress effects in these measures warrants further research. Also, whether our findings are generalizable to populations other than mostly normal-weight healthy young men and women such as obese individuals remains unclear. Indeed, appetite and satiety hormones are suggested to be differentially regulated in obese individuals (Alhabeeb et al., 2021) and potential stress effects need to be elucidated in this context. In addition, future studies are also needed to examine psychological variables that may affect the effects of stress on eating behavior such as restrained eating (Kandiah et al., 2008; Newman et al., 2007; Wallis & Hetherington, 2009; Zellner et al., 2006), emotional eating (Newman et al., 2007; Oliver et al., 2000; Raspopow et al., 2014; van Strien et al., 2012;

van Strien et al., 2014; Wallis & Hetherington, 2009), or stress eating (Epel et al., 2004; Kistenmacher et al., 2018; Sproesser et al., 2014). Finally, in our study, participants were required to start eating 15 min after stress cessation to elucidate eating during cortisol peak stress reactivity. Testing of later effects of stress on eating after cessation of the cortisol response remains to be elucidated.

Taken together, we found evidence for a lower immediate total cortisol stress reactivity relating to higher perceived hunger but not to food intake or the satiety hormone CCK. Further CCK levels of women continuously decreased while those of men were more reactive to acute stress and eating after stress. Given the observed lower cortisol levels in women (main effect of sex), the negative association between immediate total cortisol output in reaction to stress and hunger together with the observed sex differences in the satiety hormone CCK (with constantly decreasing levels of the satiety hormone CCK in women) may point to a potential mechanism underlying the higher risk for stress-induced eating in women. Future studies are needed to further elucidate and to better understand the endocrine underpinnings of stress-related obesity risk, especially in women.

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Data Availability: Data available on reasonable request from the authors.

Empirical Study 3

Acute Stress-Induced Blood Lipid Reactivity in Hypertensive and Normotensive Men and Prospective Associations with Future Cardiovascular Risk

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Abstract

Hyperreactivity to stress may be one explanation for the increased risk of cardiovascular disease (CVD) in individuals with essential hypertension. We investigated blood lipid reactivity to the Montreal Imaging Stress Task (MIST), a psychosocial stressor, in hypertensive and normotensive men and tested for prospective associations with biological risk factors. Fifty-six otherwise healthy and medication-free hypertensive and normotensive men underwent the MIST. We repeatedly measured cortisol and blood lipid profiles (total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and triglycerides [TG]) immediately before and up to 1 hr after stress. Lipid levels were corrected for stress hemoconcentration. Thirty-five participants completed follow-up assessment 2.9 ± 0.12 (*SEM*) years later. CVD risk was assessed by prospective changes in TC/HDL-C ratio, IL-6, D-dimer, and HbA1c from baseline to follow-up. The MIST induced significant changes in all parameters except TC (*p*s ≤ .043). Compared with normotensives, hypertensives had higher TC/HDL-C ratio and TG (*p*s ≤ .049) stress responses. Blood lipid stress reactivity predicted future cardiovascular risk ($p = .036$) with increases in HbA1c (ß = .34, $p = .046$), IL-6 (ß = .31, $p = .075$), and D-dimer (ß = .33, $p = .050$). Our results suggest that the greater blood lipid reactivity to psychosocial stress in hypertensives, the greater their future biological CVD risk. This points to lipid stress reactivity as a potential mechanism through which stress might increase CVD risk in essential hypertension.

Keywords: blood lipid stress reactivity; essential hypertension; Montreal Imaging Stress Task; cardiovascular risk; TC/HDL-C ratio; interleukin-6; D-dimer; hemoglobin A1c

Introduction

Cardiovascular diseases (CVD) rank among the leading causes of death in adulthood (Naghavi et al., 2017), with hypertension being one major risk factor (Whelton et al., 2018). Most hypertensive patients are diagnosed with essential hypertension as the medical cause for their chronically elevated blood pressure (BP) is unclear (Carretero & Oparil, 2000; Messerli et al., 2007). Hypertension often occurs in conjunction with other cardiovascular risk factors such as older age, obesity, insulin resistance, diabetes, and dyslipidemia (Messerli et al., 2007; Whelton et al., 2018). Especially dyslipidemia has frequently been observed in hypertensive individuals compared to normotensive controls, including increased total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) on the one hand, and decreased high-density lipoprotein cholesterol (HDL-C) on the other (Choudhury et al., 2014; Dalal et al., 2012; Gebrie et al., 2018). This adverse lipid profile is reflected by an excess proportion of atherogenic lipoproteins over HDL-C in hypertensives (Reiner et al., 2011; Sarwar et al., 2014). In particular, the TC/HDL-C ratio has been described as a suitable index reflecting the magnitude of dyslipidemia (Lemieux et al., 2001) with a strong predictive value for the incidence of both ischemic heart disease and cardiovascular risk (Calling et al., 2021; Lemieux et al., 2001).

Mental stress is an independent psychological risk factor for CVD (Kivimäki et al., 2012). According to the stress reactivity hypothesis, the study of physiological responses to controlled short-term challenges yields important insights into the complex psychobiological processes involved in the development of CVD (Linden et al., 2003). Different acute mental stressors have been shown to elicit transient elevations in atherogenic lipids and the TC/HDL-C ratio (Bachen et al., 2002; Grant et al., 2009; Radjaipour et al., 2005; Stoney, Bausserman, et al., 1999; Stoney & Finney, 2000; Wirtz et al., 2009), although only few studies found stress-induced lipid increases independent of stress hemoconcentration (Stoney, Bausserman, et al.,

1999; Stoney & Finney, 2000; Wirtz et al., 2009). This is important because hemoconcentration confounds measurement of stress-induced changes in blood lipids (Allen & Patterson, 1995). More precisely, stress can induce transient acute loss of plasma volume into the extravascular space, which results in concentration and passive increase of non-diffusible blood constituents such as lipids (Bachen et al., 2002). While studies using mild stressors did not show blood lipid increases independent of hemoconcentration (Bachen et al., 2002; Radjaipour et al., 2005), studies with more potent stressors including psychosocial threat elements did so (Stoney, Bausserman, et al., 1999; Stoney & Finney, 2000; Wirtz et al., 2009). For instance, applying the Trier Social Stress Test (TSST; Dickerson & Kemeny, 2004), which combines public speaking and mental arithmetic to be delivered in front of an audience, we found increased TC and LDL-C responses in hypertensives compared to normotensives (Wirtz et al., 2009). These results suggest hyperreactivity of atherogenic lipids to stress in hypertensives. Similar to the TSST, the Montreal Imaging Stress Task (MIST) is a psychosocial stressor that comprises motivated performance with social evaluative threat and uncontrollability (Dedovic et al., 2005). So far, the MIST has been shown to reliably induce substantial increases in salivary cortisol indicative of hypothalamic-pituitary-adrenal (HPA-) axis activation as well as increases in blood pressure and heart rate indicating sympathoadrenal medullary (SAM) arousal (Dedovic et al., 2005; Winzeler et al., 2017). Thus far, studies on blood lipid reactivity using the MIST have not been performed.

The responsivity of blood lipids to an acute standardized stressor might contribute to pathophysiological processes involved in the development of CVD. For instance, greater lipid responses to acute mild stress predicted higher TC/HDL-C ratios several years later in London-based civil servants, although stress hemoconcentration effects were not accounted for (Steptoe & Brydon, 2005). To our best knowledge, there are no studies that have investigated prospectively the effects of blood lipid stress reactivity on intermediate biomarkers of an

increased CVD risk, reflecting low-grade systemic chronic inflammation (interleukin [IL]-6; Ridker et al., 2000), a prothrombotic state (fibrin D-dimer; Willeit et al., 2013), or a diabetic condition (glycosylated hemoglobin A1c, HbA1c; Selvin et al., 2010).

We aimed to investigate (1) whether acute psychosocial stress induced by the MIST would induce changes in blood lipid levels independently of hemoconcentration in hypertensive and normotensive participants. We hypothesized that hypertensives would show greater stress-induced increases in TC, LDL-C, TG, and the TC/HDL-C ratio, but reduced HDL-C compared with normotensives. Moreover, (2) to shed light on the potential clinical relevance of higher blood lipid stress reactivity, we investigated whether reactivity of the TC/HDL-C ratio to the MIST would predict IL-6, D-dimer, and HbA1c levels after a follow-up of 2 to 5 years. We hypothesized higher blood lipid stress reactivity to predict future increases in these biomarkers of atherothrombotic CVD risk.

Materials and Methods

Study Participants

The current investigation is part of a series of studies assessing psychoneurobiological mechanisms in essential hypertension (Zuccarella-Hackl, von Känel, Thomas, Hauser, et al., 2016; Zuccarella-Hackl, von Känel, Thomas, Kuebler, et al., 2016) approved by the ethics committee of the State of Bern, Switzerland. All participants provided written informed consent and were financially compensated with CHF 140.

With the aid of the Swiss Red Cross of the State of Bern, we recruited apparently healthy, nonsmoking hypertensive and normotensive men of comparable age. In detail, members of our study team accompanied the mobile blood-donation unit of the Swiss Red Cross that routinely records BP before blood donation. Blood donors interested in the study participation were given written information asking for the following inclusion criteria: age

between 18 and 80 years; BP either in the hypertensive or in the normotensive range (see below); nonsmoker (less than five cigarettes per day), and no alcohol or illicit drug abuse. Specific exclusion criteria were verified by telephone interview using an extensive health questionnaire (Zuccarella-Hackl, von Känel, Thomas, Hauser, et al., 2016; Zuccarella-Hackl, von Känel, Thomas, Kuebler, et al., 2016). Participants were not eligible to participate when they reported any current infectious disease, current use of medication, a diagnosis of heart disease, elevated cholesterol, elevated blood sugar and diabetes, liver and renal diseases, chronic obstructive pulmonary disease, rheumatic diseases, HIV, cancer, chronic or acute clinical psychiatric disorders, as well as regular strenuous physical exercise, tobacco consumption, excessive alcohol, and illicit drug abuse. Four eligible participants (one normotensive and three hypertensives) who reported regular medication intake stopped taking their medication 1 week prior to the study. Eligible hypertensive participants provided blood samples for the routine assessment of serum creatinine, calcium, sodium, and potassium to exclude potential cases with secondary hypertension. Due to technical problems, sodium, potassium, and calcium could not be analyzed in five hypertensive participants. In one of these participants, creatinine could not be measured either. No eligible hypertensive participant was diagnosed with secondary hypertension. We measured HbA1c and blood lipids (see below) in all participants.

Classification of Essential Hypertension and Normotension

Classification of essential hypertension and normotension was based on a two-step assessment procedure. In step 1, following written instructions, interested blood donors were asked to measure their BP on 3 days at home using sphygmomanometry (Omron M6; Omron Healthcare Europe B.V., Hoofdorp, Netherlands). Home BP was to be self-measured once in the morning and once in the evening in a seated position, each after a 15-min rest. Participants were recruited as hypertensive if the average home systolic blood pressure (SBP) was $\geq$ 135

mmHg and/or the average home diastolic blood pressure (DBP) was ≥ 85 mmHg according to recommendations for home BP measurements (Williams et al., 2018). Correspondingly, participants were recruited as normotensives if the average home SBP was < 135 mmHg and the average home DBP was < 85 mmHg. We computed the average home BP of the six measurements obtained by each participant. Home BP measurement was provided by a total of 63 potential participants. Two participants were diagnosed with essential hypertension prior to their study participation and did not perform home measurements.

In step 2, we verified the home-measurement-based preliminary classification of each of these participants as hypertensive or normotensive. Trained personnel performed three additional BP measurements during the first study session with the participant in a seated position after a 15-min rest. We applied the regular World Health Organization (WHO)/International Society of Hypertension (ISH) definition of hypertension and classified medication-free participants as hypertensive if their average study SBP was ≥ 140 mmHg and/or their average study DBP was ≥ 90 mmHg (Chalmers et al., 1999). Participants were classified as normotensive if their average study SBP was < 140 mmHg and their average study DBP was < 90 mmHg.

The final group assignment of medication-free participants was based on congruent home and study BP classification and comprised 28 normotensives and 28 essential hypertensives. Six participants were excluded due to normotensive home, but hypertensive study BP (white coat hypertension), and three participants were excluded due to hypertensive home and normotensive study BP (masked hypertension).

Design and Procedure

Cross-sectional data assessment comprised 2 days. On the 1st study day, we performed study BP classification to verify the home-measurement-based preliminary classification of hypertension and normotension and to assess medical information. Hypertensive and

normotensive participants with congruent home and study BP classification were then invited to a 2nd study day to undergo a standardized stress test.

First Study Day and Baseline CVD Risk Assessment. All participants consumed a semistandardized breakfast following written instructions and abstained from caffeine and alcohol consumption for 24 hr prior to their arrival at the lab at 8:00. After providing written informed consent, participants´ body measurements (height and weight) were assessed. Resting study BP was assessed three times by means of sphygmomanometry (Omron M6; Omron Healthcare Europe B.V., Hoofdorp, Netherlands), each after a 15-min rest period. Blood samples were collected at 11:30, i.e., after a fasting for 3.5 hr since arrival. Due to technical problems, baseline blood sampling was missing in one hypertensive participant and incomplete in one normotensive participant.

Second Study Day and Stress Reactivity Assessment. All participants were instructed to abstain from food for at least 1 hr, and from alcoholic and caffeinated beverages and strenuous exercise for at least 24 hr before the second study appointment. They were further required to be well rested and to have maintained a regular sleep-wake rhythm for 3 nights before the reactivity testing. Participants arrived between 12:00 and 13:00 at the lab, where they received detailed explanations of the testing procedure and provided informed consent for the 2nd study day. Then, trained study nurses inserted an intravenous catheter into the participants' nondominant forearm for blood sampling. During the following 45-min acclimatization period, participants completed psychosocial questionnaires ahead of the collection of baseline blood samples. Afterwards, participants underwent a standardized psychosocial laboratory stressor for imaging studies (i.e., the MIST) as explained below. The recovery period started after cessation of the stressor and lasted for 1 hr. Blood and saliva samples were collected immediately before stress induction by the MIST (−1 min) and repeatedly after MIST cessation (+1 min, +10 min, +20 min, and +60 min). Additional saliva

samples were assessed 30 and 45 min after stress. Participants were in supine position during the experimental procedure, and all blood samples were drawn in supine position. Participants only raised to move from the test room to the nearby scanner room (after MIST baseline blood sample) and back again (after blood sample +1 min). They were also unrestricted to visit the restrooms at any time.

Assessment of CVD Risk at Follow-Up. To assess longitudinal changes in CVD risk factors, we invited our study participants to a follow-up assessment at least 2 years later for blood sampling procedures identical to the 1st study day. All 56 participants of the baseline assessment were invited for the follow-up assessment scheduled between 2 and 5 (M = 2.86 ± 0.12) years later, with a final sample of 35 participants completing both assessments. Reasons for drop-out comprised severe illness (n = 4), no interest (n = 2), not reachable by phone (n = 5), lack of time (n = 5), excessive demand (n = 1), or no reason given (n = 4). Notably, we initially intended to schedule for a 2-year follow-up period. However, due to the relocation of the working group from Bern (Switzerland) to Konstanz (Germany), the follow-up assessments in Bern were hampered by limited personnel resources and logistic challenges. This resulted in substantially longer follow-up intervals with potential effects on drop-out rates.

Montreal Imaging Stress Task (MIST)

Psychosocial stress was induced following the standard protocol of the MIST. This standardized stress paradigm was developed for functional imaging studies and reliably induces psychophysiological stress responses (Dedovic et al., 2005). The MIST is based on the Trier Mental Challenge Task, and combines a series of computerized mental arithmetic challenges with social evaluative threat components (Pruessner et al., 1999). The MIST was carried out with (stress condition) or without (control condition) time pressure and social evaluation.

In detail, starting with a training session, participants' ability to perform mental arithmetic was assessed outside the scanner, and the average time needed to solve problems was used to set a default time limit for the experimental condition. In both conditions (stress and control), participants had to solve mental arithmetic tasks presented on a projection screen, which additionally displayed performance feedback (i.e., correct, incorrect, or time out). Stress was induced by consistently adapting the difficulty and time provided to solve the mental arithmetic to ensure a 50% to 60% failure rate. Simultaneously, a continuous performance progress bar on the screen indicated to the participants that their performance was weaker compared to that of all other participants of similar age, educational level, and professional position. Between runs, two confederates, introduced as study investigator and as study leader, enforced social evaluative threat by giving standardized fake feedback (Dedovic et al., 2005).

As first feedback, the study investigator reminded the participant that there is a required minimum performance for the participant to be used in the analysis of the functional scan. The investigator informed the participant that the scanning session had to be stopped because of his poor performance. The investigator also asked about potential reasons for this poor performance (e.g., problems with understanding the task, problems with the response box, or visual problems with the screen). Finally, the participant was told that the study investigator, the study leader, and the MR technicians were following his performance on a second monitor in the control room of the scanning environment and asked him to give his best to provide useful data. The investigator then left the scanner room, and the next run of mental arithmetic was initiated.

The second feedback was given by the supposed leader of the study. The latter informed the participant that his performance had been monitored and that the scanning session had to be stopped again because of his poor performance. The study leader asked the participant if he was aware of his below-average performance. He reminded the participant that his performance

should at least approach the average user level if his data were to be used in the study. Since he had often exceeded the time provided to solve the mental arithmetic (as indicated by "timeout"), the participant was also asked about potential problems with concentration in the last days or any alcohol intake during the last 24 hr. Finally, the participant was asked to try his best to provide useful data; the investigator further explained that useful data were naturally the primary goal of the whole study team since a lot of energy and money had already been spent on the participants' fMRI data acquisition. Then, the third run of mental arithmetic was initiated.

The control condition consisted of mental arithmetic tasks with comparable difficulty level but presented without any time restriction and/or negative feedback. Individual and average users' performance were not displayed.

In total, the participants underwent three runs. In each run, the stress (105 s) and the non-stress (45 s) conditions were presented in a blocked design with three repetitions in a counterbalanced order. The two feedbacks were given between runs 1 and 2 (feedback 1), and between runs 2 and 3 (feedback 2). The total duration of the MIST was about 30 min.

Biochemical Analyses

Blood Lipids. Blood lipid profiles were assessed on all 3 study days. On study day 2, measurements were immediately before and after stress, as well as 10, 20, and 60 min after stress cessation (−1 min, +1 min, +10 min, +20 min, and +60 min). We repeatedly measured total TC and HDL-C to allow computation of TC/HDL-C ratio (Lemieux et al., 2001), as well as TG from heparine-coated monovettes (Sarstedt monovette orange). Analyses were performed at the Center for Laboratory Medicine of the Bern University Hospital (Inselgruppe AG, Bern, Switzerland) using in vitro assays (enzymatic colorimetric) for the quantitative determination of blood lipids in human plasma (Roche, Mannheim, Germany) on a Roche/Hitachi Cobas C Analyzer (Roche, Mannheim, Germany). LDL-C was calculated using

the Friedewald formula: LDL-C = TC − HDL-C − (TG/2.19). Mean inter- and intra-assay CVs were ≤ 1.2% and ≤ 2.5%, respectively. In order to correct plasma lipid levels for stress hemoconcentration, we additionally assessed hemoglobin (grams per deciliter) and hematocrit (percentage) by processing whole blood collected in 2.7 ml EDTA-coated tubes (Sarstedt, Nümbrecht, Germany) on an automated hematology system (Advia 120, Siemens Diagnostics, Erlangen, Germany) at the Center for Laboratory Medicine. Due to technical problems with blood sampling, baseline (i.e., day 1) blood lipids were missing in one hypertensive and one normotensive participant.

Cortisol. We measured cortisol as a manipulation check to test whether the MIST successfully induced cortisol stress reactivity as observed in previous research (Dedovic et al., 2005). For assessment of cortisol, saliva samples were collected at 7 timepoints (−1 min, +1 min, +10 min, +20 min, +30 min, +45 min, and +60 min) using salivette devices (Sarstedt, Rommelsdorf, Germany), which were stored at −20 °C until biochemical analysis. Prior to analyses, saliva samples were thawed and spun at 3000 rpm for 10 min, yielding low-viscosity saliva. Cortisol concentrations were measured using a commercially available competitive chemiluminescence immune assay with high sensitivity of 0.16 ng/ml (LIA, IBL Hamburg, Germany). Intra- and inter-assay CVs were < 7.7% and 11.5%, respectively. Cortisol assessment was missing in one hypertensive participant, and cortisol data of another normotensive participant were not considered due to an outlier value (> 70 nmol/L) at baseline.

CVD Risk Assessment. CVD risk was assessed in all participants at baseline and prospectively at follow-up. We assessed CVD risk by measurement of the following risk factors: TC/HDL-C ratio, the hypercoagulability marker D-dimer, HbA1c, as well as the pro-inflammatory cytokine IL-6. Blood lipids, D-dimer, and HbA1c were analyzed at the Center for Laboratory Medicine of the Bern University Hospital (Inselgruppe AG, Bern, Switzerland),

while IL-6 analyses were performed in the biochemical laboratory of the Biological Work and Health Psychology group at the University of Konstanz.

For assessment of D-dimer, venous blood was drawn into polypropylene tubes containing 3.8% sodium citrate (Sarstedt, Nümbrecht, Germany). Citrate tubes were immediately centrifuged for 20 min at 4 °C at 2000× g, and plasma was pipetted into aliquots. D-dimer was analyzed using a particle-enhanced immunoturbidimetric assay for the quantitative determination of D-dimer in human plasma (INNOVANCE® D-Dimer, Siemens Healthcare GmbH, Erlangen, Germany) on a Sysmex CS-5100 (Sysmex Europe, Norderstedt, Germany). The intra- and inter-assay coefficients of variation were ≤ 7.9%.

To measure HbA1c, venous blood was drawn into EDTA-coated monovettes, and analyses were performed with in vitro assays for the quantitative determination of HbA1c IFCC (mmol/mol) in whole blood (Tina-quant®, Roche, Mannheim, Germany) using Roche/Hitachi Cobas C Systems (Roche, Mannheim, Germany). Mean inter- and intra-assay CVs were ≤ 1.6% and ≤ 2.0%, respectively.

For the determination of IL-6, venous blood was drawn in EDTA-coated monovettes (Sarstedt, Nümbrecht, Germany) and immediately centrifuged for 10 min at 2000× g and 4 °C. Obtained plasma was stored at −80 °C until analysis. IL-6 levels were determined with a high-sensitivity sandwich immunoassay (Meso Scale Discovery (MSD), Rockville, MD, USA). Mean inter- and intra-assay CVs were ≤ 7.3% and ≤ 4.5%, respectively, and the detection limit was 0.06 pg/ml. Baseline measurements were incomplete in one hypertensive and one normotensive participant (see Table 3.1 for details).

Statistical Analyses

Statistical analyses were performed using SPSS (Version 26.0) statistical software packages for MacIntosh (IBM SPSS Statistics, Chicago, IL, USA). All tests were two-tailed with level of significance at $p < .05$ and p-values < .10 interpreted as borderline significant.

G*Power (Version 3.1.9.6; Heinrich Heine University Düsseldorf, Germany) analysis suggests that a total sample size of $N = 54$ is needed to detect group differences in plasma lipid stress reactivity (five repetitions) with a small-to-medium effect size of $f = 0.15$ in general models with repeated measures with a power of .85, $\alpha = .05$, given the previously observed minimum intercorrelation among repeated measures of .63 and $\varepsilon = .79$ (Kuebler, Trachsel, et al., 2014).

We corrected all plasma lipid levels for stress hemoconcentration following previous methods by computing stress-induced changes in plasma volume (i.e., stress hemoconcentration) from hemoglobin and hematocrit measures according to the formula by Dill and Costill (Austin et al., 2012; Dill & Costill, 1974). Body mass index (BMI) was calculated by the formula BMI = kg/m^2.

Univariate analyses of variance (ANOVAs) were used to compute group differences in participant characteristics. To test for MIST-induced increases in the studied measures, we calculated ANOVAs with repeated measures for cortisol (baseline, 1 min, 10 min, 20 min, 30 min, 45, min, and 60 min post-stress) and blood lipids (baseline, 1 min, 10 min, 20 min, and 60 min post-stress) over all participants. Significant changes of each measurement from baseline were identified post hoc.

As main analyses, we first investigated whether hypertensives exhibited higher blood lipid increases to acute stress compared with normotensives. We calculated repeated-measures ANCOVAs with repeated assessment of hemoconcentration-corrected blood lipids (baseline, 1 min, 10 min, 20 min, and 60 min post-stress) as dependent variables and with group as the independent variable. Repeated measures ANCOVAs were also calculated to examine possible group differences in cortisol (baseline, 1 min, 10 min, 20 min, 30 min, 45, min, and 60 min post-stress). We controlled for age and BMI as covariates in the repeated-measures ANCOVAs. Post hoc testing of significant stress effects in cortisol and blood lipid measures

comprised univariate ANOVAs with group as the independent variable and the respective single measurements as dependent variables.

We performed prospective analyses to shed light on the potential clinical relevance of blood lipid stress reactivity and analyzed whether blood lipid stress responses would predict future changes in CVD risk factors. We calculated multiple analyses of variance (MAN(C)OVA) with aggregated blood lipid stress reactivity calculated as area under the curve with respect to ground (AUC_G; Pruessner et al., 2003) as independent variable. Dependent variables were prospective changes in TC/HDL-C ratio, D-dimer, HbA1c, and IL-6 from baseline to follow-up assessment (follow-up measurement of the respective parameter minus its baseline measurement) to investigate CVD risk as dependent variable. Baseline age, MAP, and time between baseline and follow-up assessments were controlled as a set of covariates in complementary analyses, in addition to BMI, prospective changes in BMI and MAP, or medication intake at follow-up assessment. Post hoc testing comprised linear regression analyses with the aggregated blood lipid stress reactivity (AUC_G) as independent variable and changes in CVD risk factors separately (i.e., changes in TC/HDL-C ratio, D-dimer, HbA1c, and IL-6) as dependent variables, while controlling for the set of confounders, i.e., covariates.

All data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests prior to statistical analyses. All measures showing a skewed distribution (home MAP and SBP, stress reactivity of cortisol and most TG levels, HbA1c, IL-6, and D-dimer at baseline as well as prospective changes in TC/HDL-C ratios, HbA1c, IL-6, BMI, and MAP) were log-transformed. While log-transformed data were used for modeling and testing, we depict untransformed data in all Tables and Figures for reasons of clarity. Blood lipids stress reactivity was depicted as (percentage) changes from baseline to post-stress.

We applied Huynh-Feldt correction for repeated measures where appropriate. We determined *f* from partial η^2 values using G*Power. Effect size parameters *f* (effect size conventions *f*: 0.10 = small; 0.25 = medium; 0.40 = large) and R^2 changes (effect size conventions ΔR^2: .02 = small; .13 = medium; .26 = large) are reported where appropriate (Cohen, 1988).

Results

Group Characteristics

Table 3.1 shows the demographic, medical, and psychological characteristics of the 28 hypertensive and 28 normotensive participants. As expected, hypertensives showed higher systolic and diastolic BP as well as MAP, higher BMI, IMT, IL-6, and fasting TG, as well as higher LDL-C/HDL-C and TC/HDL-C ratio at baseline (i.e., on the 1st study day) compared with normotensives (*p*s ≤ .010).

Hypertensives had also a higher MIST baseline TC/HDL-C ratio than normotensives ($p = .023$) did, but there were no other differences in MIST baseline blood lipid levels (*p*s ≥ .11; see Table 3.2). At follow-up, eight of the initially medication-free participants were under medication: five hypertensives and one normotensive took BP-lowering medication, one hypertensive took cholesterol-lowering medication, and another hypertensive participant took uric acid-lowering medication.

Participants who dropped out ($n = 21$) did not significantly differ in any group characteristic (*p*s ≥ .15; see Appendix, Table 3.A1) from participants completing both baseline and follow-up assessment ($n = 35$).

Table 3. 1: Comparison of Baseline Group Characteristics Between Hypertensive and Normotensive Participants

	Hypertensives (*n* = 28)	Normotensives (*n* = 28)	*p*
Age, years	49.82 ± 2.04 (25–74)	49.75 ± 2.22 (28–74)	.98
BMI, kg/m^2	28.29 ± 0.64 (22.67–35.25)	24.99 ± 0.55 (19.78–30.85)	< .001***
Home BP, mmHg			
Home MAP	106.85 ± 1.17 (95.61–121.50), *n* = 26	87.21 ± 0.92 (75.06–94.72)	< .001***
Home SBP	144.83 ± 1.82 (125.67–162.33), *n* = 26	121.08 ± 1.19 (105.17–130.50)	< .001***
Home DBP	87.85 ± 1.28 (78.33–103.00), *n* = 26	70.27 ± 1.011 (60.00–79.33)	< .001***
Study BP, mmHg			
Study MAP	115.96 ± 1.84 (103.50–139.89)	93.88 ± 1.41 (75.33–104.56)	< .001***
Study SBP	153.70 ± 2.65 (129.33–189.67)	127.39 ± 1.67 (109.33–139.67)	< .001***
Study DBP	97.08 ± 1.58 (84.67–115.00)	77.13 ± 1.46 (58.33–87.67)	< .001***
TG, mmol/L	1.52 ± 0.12 (0.77–3.76), *n* = 27	1.10 ± 0.09 (0.36–2.35), *n* = 27	.007**
TC, mmol/L	5.67 ± 0.16 (4.11–7.18), *n* = 27	5.22 ± 0.23 (3.30–7.79), *n* = 27	.12
LDL-C, mmol/L	3.91 ± 0.16 (2.33–5.19), *n* = 27	3.48 ± 0.22 (1.73–6.53), *n* = 27	.11
HDL-C, mmol/L	1.43 ± 0.07 (0.93–2.22), *n* = 27	1.59 ± 0.06 (0.92–2.21), *n* = 27	.11
LDL-C/HDL-C	2.88 ± 0.16 (1.32–4.32), *n* = 27	2.26 ± 0.16 (1.05–4.35), *n* = 27	.010*
TC/HDL-C	4.14 ± 0.19. (2.38–5.77), *n* = 27	3.41 ± 0.19 (2.01–5.49), *n* = 27	.009**
HbA1c, mmol/mol	35.59 ± 0.82 (26–43), *n* = 27	36.59 ± 0.57 (31–42), *n* = 27	.28
D-dimer, µg/L	375.37 ± 40.73 (45–955), *n* = 27	440.82 ± 72.56 (45–1616)	.77
IL-6, pg/ml	0.63 ± 0.07 (0.16–1.85), *n* = 27	0.37 ± 0.03 (0.15–0.76)	< .001***
Time between baseline and follow-up assessments, months	40.75 ± 2.14 (28–61)	39.33 ± 1.81 (28–63)	.62
Creatinine, µmol/L	80.48 ± 1.63 (66–93), *n* = 27		
Sodium, mmol/L	140.39 ± 0.50 (135–145), *n* = 23		
Calcium, mmol/L	2.40 ± 0.02 (2.17–2.58), *n* = 23		
Potassium, mmol/L	4.11 ± 0.04 (3.9–4.7), *n* = 23		

Note. Values are means ± *SEM* (range); BMI = body mass index; BP = blood pressure; MAP = mean arterial blood pressure; DBP = diastolic blood pressure; SBP = systolic blood pressure; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; HbA1c = hemoglobin A1c; IL-6 = interleukin-6. Deviating participant numbers are indicated. * $p < .05$; ** $p < .01$; *** $p < .001$.

Cortisol and Blood Lipid Reactivity to the MIST in all Participants

Cortisol. As a manipulation check, we tested whether the MIST successfully induced cortisol stress reactivity as observed in previous research (Dedovic et al., 2005; see Figure 3.1A). Across all participants, the MIST induced significant increases in cortisol (main effect

of stress: $F(2.88, 152.86) = 70.39$, $p < .001$, partial $\eta^2 = .57$, $f = 1.15$), with highest levels observed 10 min after MIST cessation. Post hoc testing revealed significant increases from MIST baseline to 1 min ($p < .001$), 10 min ($p < .001$), 20 min ($p < .001$), 30 min ($p < .001$), and 45 min ($p < .001$) after MIST cessation.

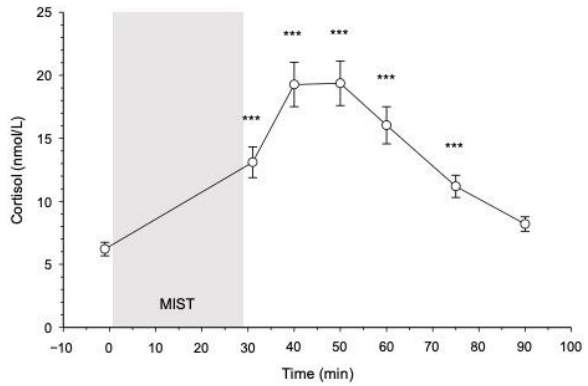
Table 3. 2: Comparison of MIST Baseline Levels of Cortisol and Blood Lipids in Hypertensives and Normotensives

	Hypertensives (n = 28)	Normotensives (n = 28)	p
Cortisol, nmol/L	7.22 ± 0.92 (1.83–17.85)	5.21 ± 0.50 (1.61–12.50)	.16
HDL-C, mmol/L	1.25 ± 0.06 (0.87–1.93)	1.35 ± 0.05 (0.86–1.83)	.20
LDL-C, mmol/L	3.31 ± 0.15 (1.63–4.51)	2.95 ± 0.16 (1.36–5.37)	.11
TG, mmol/L	1.66 ± 0.14 (0.61–3.61)	1.38 ± 0.11 (0.35–2.87)	.12
TC/HDL-C	4.14 ± 0.19 (2.46–6.29)	3.54 ± 0.17 (2.13–5.65)	.023*

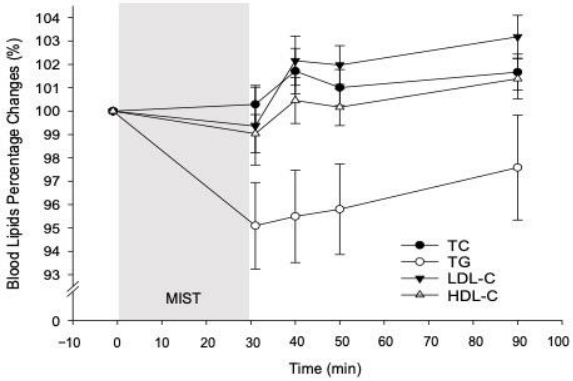
Note. Values are means ± *SEM* (range); HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; TG = triglycerides; TC = total cholesterol. * $p < .05$.

Blood Lipids. As shown in Figure 3.1B, the MIST induced in all participants significant increases in HDL-C (main effect of stress: $F(3.50, 192.70) = 2.62$, $p = .043$, partial $\eta^2 = .05$, $f = 0.22$) and LDL-C levels (main effect of stress: $F(2.95, 161.96) = 3.53$, $p = .017$, partial $\eta^2 = .06$, $f = 0.25$), while increases in TC levels did not reach statistical significance ($p = .13$). In contrast, TG levels decreased in all participants (main effect of stress: $F(2.0, 109.70) = 5.01$, $p = .008$, partial $\eta^2 = .08$, $f = 0.30$). The TC/HDL-C ratio increased after stress, with highest ratios immediately after stress cessation (main effect of stress: $F(2.46, 135.38) = 6.62$, $p < .001$, partial $\eta^2 = .11$, $f = 0.35$; see Figure 3.1C). Compared to baseline, post hoc testing revealed significant increases in TC/HDL-C ratio immediately ($p = .017$) and 10 min after stress ($p = .028$), whereas TG levels decreased immediately after stress cessation ($p = .044$).

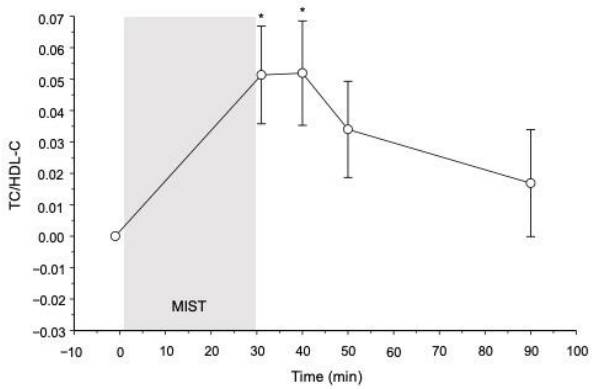
Figure 3. 1: Cortisol and Blood Lipid Reactivity to the MIST Over All Participants



(**A**)



(**B**)



(**C**)

Note. (A-C). Cortisol and blood lipid changes in response to the MIST. Values are means ± *SEM*. (A) Cortisol. (B) Percentage changes from baseline to post-stress in TC, TG, LDL-C, and HDL-C. (C) TC/HDL-C ratio changes from baseline in response to MIST. Asterisks indicate significant differences between baseline and the respective post-stress levels * $p < .05$; *** $p < .001$.

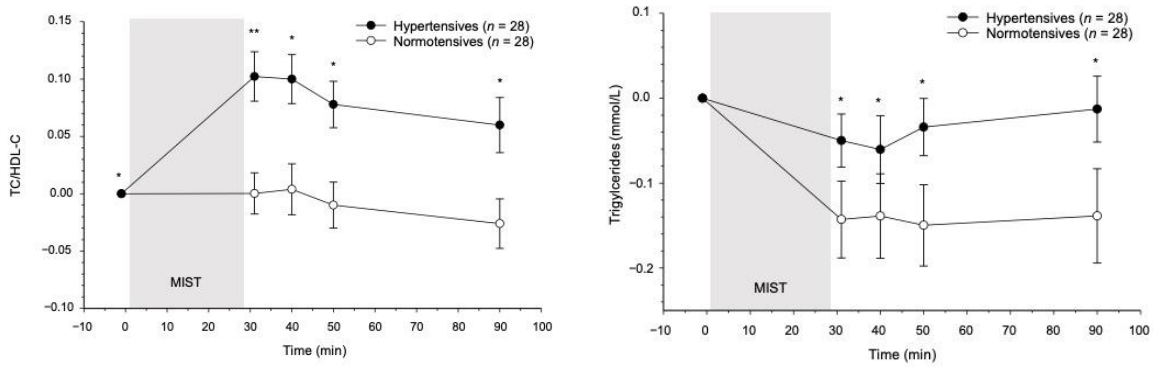
Reactivity to the MIST in Hypertensives as Compared to Normotensives

Baseline. At MIST baseline (see Table 3.2), hypertensives had higher TC/HDL-C ratio ($p = .023$) but did not differ in cortisol ($p = .16$) or other blood lipid ($ps \geq .11$) levels.

Reactivity. In terms of blood lipids, hypertensives and normotensives significantly differed in the stress response of the TC/HDL-C ratio (interaction group-by-time: $F(2.79, 145.41) = 4.09$, $p = .010$, partial $\eta^2 = .07$, $f = 0.28$). Hypertensives showed a higher TC/HDL-

C ratio in response to stress compared with normotensive men (see Figure 3.2A and Table 3.A2) at all post-stress measurements (+1 min: p = .009; +10 min: p = .010; +20 min: p = .010; +60 min: p = .011). Complementary analyses using MAP instead of group confirmed higher TC/HDL-C ratio stress reactivity with increasing MAP (interaction MAP-by-time: F(2.73, 141.76) = 2.25, p = .091, partial η^2 = .04, f = 0.21). The two groups differed also in their stress-induced TG reactivity (interaction group-by-time: F(2.15, 111.59) = 3.02, p = .049, partial η^2 = .06, f = 0.24) (see Figure 3.2B). Compared with normotensives, hypertensives showed higher TG levels (Figure 3.2B) (+1 min: p = .034; +10 min: p = .027; +20 min: p = .017; +60 min: p = .013) and thus a lower decline in TG levels after stress cessation. The reactivity difference in TG between hypertensives and normotensives was confirmed in complementary analyses using MAP as continuous dependent variable (interaction MAP-by-time: F(2.14, 111.37) = 4.36, p = .013, partial η^2 = .08, f = 0.29). Stress-induced reactivity in TC (p = .71), in HDL-C (p = .65), and in LDL-C (p = .60) did not differ between groups. There were no group differences in terms of cortisol reactivity to the MIST (p = .12).

Figure 3. 2: Blood Lipid Reactivity to MIST in Hypertensive and Normotensive Men



(**A**) (**B**)

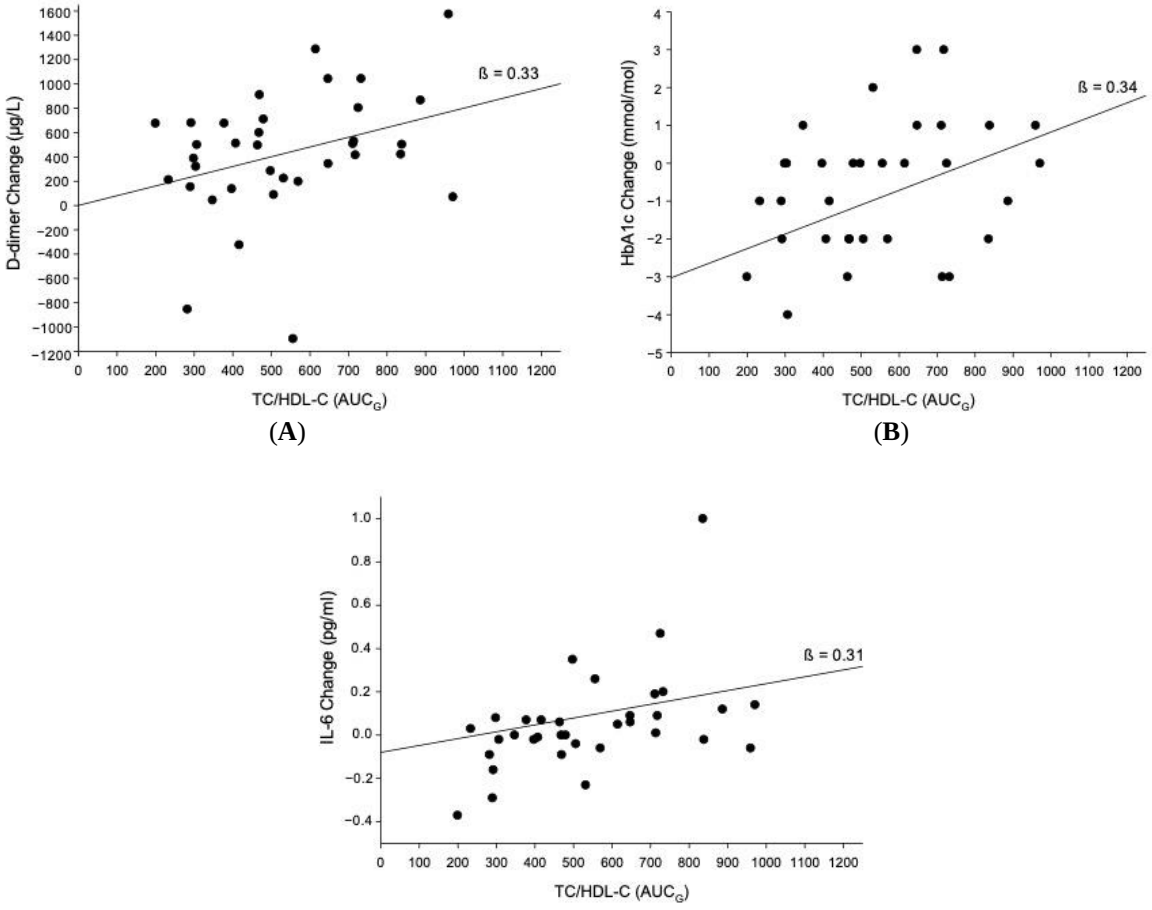
Note. Blood lipid reactivity to the MIST in hypertensive and normotensive men. Values are means ± *SEM*. (A) MIST-induced TC/HDL-C ratio changes from baseline. (B) MIST-induced TG changes from baseline. * p < .05; ** p < .01.

Prediction of CVD Risk Factors

MANOVA revealed that higher aggregated TC/HDL-C stress reactivity (AUC_G) significantly predicted increased CVD risk in terms of increases in the measured CVD risk factors from baseline to follow-up (main effect in multivariate testing: $F(4, 30) = 2.95$, $p = .036$, partial $\eta^2 = .28$, $f = 0.62$, Wilk's $\Lambda = .72$). Complementary MANCOVA analyses controlling for age, MAP, and time between baseline and follow-up assessments as covariates confirmed the main effect of TC/HDL-C stress reactivity as a predictor of increased CVD risk over time ($F(4, 27) = 3.35$, $p = .024$, partial $\eta^2 = .33$, $f = 0.70$, Wilk's $\Lambda = .67$). Additional controlling for BMI, changes in BMI and MAP over time, or medication-intake at follow-up did not alter the significance of this main effect ($ps \leq .029$).

Post hoc testing revealed that higher blood lipid stress reactivity in terms of TC/HDL-C AUC_G predicted greater increases from baseline to follow-up in D-dimer (without control: ß = .33, $p = .050$, $\Delta R^2 = .11$, Figure 3.3A; controlling for the set of covariates: $p = .11$; additional controlling for medication intake, BMI, BMI change, or MAP change: $ps \geq .09$), HbA1c (without control: ß = .34, $p = .046$, $\Delta R^2 = .12$, Figure 3.3B; controlling for the set of covariates: ß = .32, $p = .32$, $\Delta R^2 = .15$; additional controlling for medication intake, BMI, BMI change, or MAP change: $ps \geq .32$), and IL-6 (without control: ß = .31, $p = .075$, $\Delta R^2 = .09$, Figure 3.3C; controlling for the set of covariates: ß = .45, $p = .019$, $\Delta R^2 = .24$ additional controlling for medication intake, BMI, BMI change, or MAP change: ßs ≥ .43, $ps \leq .026$, $\Delta R^2 \geq .24$). Prediction of higher TC/HDL-C increases was not significant ($p = .57$) but became of borderline significance when controlling for the set of covariates (ß = .23, $p = .064$, $\Delta R^2 = .58$; additional controlling for medication intake, BMI, BMI change, or MAP change: ßs ≥ .25, $ps \leq .073$, $\Delta R^2 \geq .59$).

Figure 3. 3: Changes in CVD Risk Factors Predicted by TC/HDL-C Stress Reactivity



Note. Higher aggregated TC/HDL-C stress reactivity (AUC_G) significantly predicted higher increases from baseline to follow-up in CVD risk factors: (A) D-dimer (ß = .33, p = .050, ΔR^2 = .11); (B) HbA1c (ß = .34, p = .046, ΔR^2 = .12); (C) IL-6 (ß = .31, p = .075, ΔR^2 = .09).

Discussion

The main objective of our study was to investigate whether acute psychosocial stress induced by the MIST would relate to changes in blood lipid levels (i.e., TC, HDL-C, LDL-C, TG, and TC/HDL-C ratio) with expected reactivity differences between hypertensive and normotensive participants. We were further interested in the clinical relevance of blood lipid stress responses and tested whether TC/HDL-C stress reactivity would prospectively predict changes in CVD risk factors.

Our results confirm that stress induction by means of the MIST was successful, as indicated by salivary cortisol increases in line with previous MIST studies (Dedovic et al., 2005). Moreover, we could extend the stress-provoking potential of the MIST for the first time to blood lipids. Independent of hemoconcentration, we observed significant increases in HDL-C, LDL-C, as well as in TC/HDL-C ratio scores in response to the MIST. The observed increases in atherogenic lipids are in line with previous studies using other potent stressors while correcting for hemoconcentration effects (Stoney, Bausserman, et al., 1999; Stoney & Finney, 2000; Wirtz et al., 2009). Interestingly, in our study, TG levels decreased after stress induction. Because triglycerides serve as energy reserves, we assume that the decrease in triglycerides after stress may represent a process of energy supply in reaction to stress-induced increased energy expenditure (Hitze et al., 2010).

With respect to group differences, hypertensives showed a more pronounced rise in TC/HDL-C ratio scores and a slower decrease in TG levels in response to the MIST compared with normotensive participants. Complementary analyses using MAP confirmed the linear nature of this effect. The observed stress-related changes in atherogenic lipids resemble our previous observations in a TSST study (Wirtz et al., 2009). We interpret our findings to mean that with increasing BP, hypertensives seem to be more vulnerable to acute psychosocial stress in terms of blood lipid responses. Group differences in stress-induced blood lipid reactivity might be elicited by the metabolic effect of catecholamine spillover in sympathetic nerve endings and from the adrenal medulla in response to acute psychosocial stress (Arner, 2005; Wirtz et al., 2009). Increases in circulating catecholamines can induce lipolysis and release free fatty acids into the circulation (Arner, 2005). With respect to cortisol analyzed in our study, stress induction by means of the MIST did not result in group differences between hypertensives and normotensives as observed in our previous TSST study (Wirtz, von Känel, et al., 2006). We assume that the differences in stress-evoking components between the MIST

and the TSST may account for this discrepancy. We performed the MIST in an MRT scanner, and the MRT experience may constitute part of the MIST stress experience that is not psychosocial in nature. Moreover, the direct contact with the experimenter is lower in the MRT scanner-based MIST as compared to the TSST with its permanent social confrontation. If the cortisol stress hyperreactivity observed in hypertensive participants mainly results from psychosocial stress aspects, their reactivity to the MIST might be comparably lower. At the same time, it is possible that normotensive participants may react stronger to the MIST, especially if performed within the potentially intimidating surrounding of an MRT scanner, as to the primarily psychosocial TSST.

Our prospective analyses were aimed to shed light on the supposed clinical relevance of blood lipid stress reactivity. Indeed, we found evidence for blood lipid stress reactivity in predicting future CVD risk in terms of increases over time in the intermediate biological risk factors HbA1c, D-dimer, and IL-6, and in part also in the TC/HDL-C ratio. Notably, one hypertensive participant started intake of cholesterol-lowering medication between baseline and follow-up assessment, and exclusion of this participant from analyses did not significantly change the observed prediction (data not shown). Given the observed higher blood lipid stress reactivity with increasing hypertension, our prospective findings suggest that blood lipid stress reactivity may play a role in mediating future CVD risk in hypertensives. We offer several possible explanations for these findings: (1) Atherosclerosis with its underlying process of chronic inflammation is central to CVD (Libby, 2021; Ross, 1999). We speculate that increases in blood lipids, even if transient, may promote atherosclerosis by acutely forcing blood lipids into the arterial wall, thus adding to lipid accumulation in the intima-media of the carotid arteries. Increased lipid accumulation in the arterial wall of carotid arteries may relate to increased amounts of oxidized lipids, which in turn promote both inflammatory and atherothrombotic processes in atherosclerosis (Libby, 2021; Ross, 1999). In line with such

reasoning, we observed higher lipid stress reactivity to predict higher prospective IL-6 and fibrin D-dimer increases. At the same time, both IL-6 and fibrin D-dimer are independent CVD risk factors that can further promote atherosclerotic processes (Ridker et al., 2000; Tyrrell & Goldstein, 2021; Willeit et al., 2013). (2) Our borderline significant finding of blood lipid stress reactivity independently predicting increased TC/HDL-C ratio over time confirms a previous study Steptoe and Brydon (2005). Extending these prior results, our data suggest that this association seems to be independent of hemoconcentration effects. In line with the allostatic load model, we speculate that repeated stressful experiences may accumulate over time to result in chronic elevations of blood lipid levels (McEwen, 1998b), but the precise underlying mechanism warrants further investigation. (3) Diabetic dyslipidemia is a well-known phenomenon, especially in the context of the metabolic syndrome clustering increased BP, high blood sugar, and dyslipidemia (Farmer, 2008). However, potential mechanisms underlying our finding of blood lipid stress responses to predict increases in HbA1c as an indicator of increased blood sugar over time are unclear. Notably, our observed associations were not independent of confounders, in particular of basal MAP, suggesting that basal BP effects may play a role in modulating the observed HbA1c increases.

Limitations of our study are the relatively small number of participants due to the experimental nature of our study and the comparably high drop-out rate that we mainly attribute to organizational reasons. While the participant number for our stress study was based on our a priori sample size calculations allowing to detect effects of small-to-medium effect sizes, the reduced sample size available for the prospective analyses only allowed to detect medium-to-large effects. We therefore cannot rule out the possibility that the study was underpowered to detect smaller effects and that our results might be biased. Notably, given our follow-up sample size, we only allowed for a maximum of four confounders in follow-up analyses (Babyak, 2004). Although participants who dropped out did not substantially differ in their

characteristics at baseline from those completing the follow-up assessment, there were more drop-outs in the hypertensive group. Furthermore, eight participants started medication intake between baseline and follow-up assessment, which could possibly influence prospective outcomes, although we controlled for potential medication effects. Finally, the generalizability of our results is limited to middle-aged normotensive and essentially hypertensive men.

Conclusions

Taken together, we observed significant changes in blood lipid levels in reaction to stress induction by means of the MIST. Blood lipid levels were higher after stress in hypertensive individuals compared with normotensive controls and also with increasing MAP. As the aggregated TC/HDL-C stress reaction predicted CVD risk factors several years later, blood lipid stress reactivity might be a potential mechanism contributing to a poorer cardiovascular health in hypertensives. Future studies are needed to replicate our cross-sectional and in particular prospective findings in larger sample sizes including women and clinical populations. Subsequent studies could further investigate whether specific medication (e.g., statin therapy) could attenuate the effects of stress on lipid levels. Moreover, the mechanisms underlying the observed stress-reactivity differences between hypertensives and normotensives and the prospective associations between blood lipid stress reactivity and CVD risk warrant further investigation.

Author Contributions: Conceptualization, PHW; formal analysis, CD; funding acquisition, PHW and RvK; investigation, LT, CZ-H, and RvK; methodology, RvK, RW, JCP, and PHW; project administration, RvK and PHW; supervision, RvK and PHW; visualization, CD and PHW; writing—original draft, CD and PHW; writing—review and editing, RvK, RW, and CZ-H. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the ethics committee Ethics Committee of the Canton of Bern, Switzerland (154/07) and the Swiss Agency for Therapeutic Products (Swissmedic) Switzerland.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not available.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix

Table 3.A 1: Comparison of Baseline Group Characteristics Between Participants Completing Follow-Up Assessment and Drop-Out Participants

	Follow-Up (n = 35)	Drop-Out (n = 21)	p
Age, years	50.20 ± 1.91 (28–74)	49.10 ± 2.45 (25–69)	.72
BMI, kg/m^2	26.37 ± 0.62 (19.78–34.58)	27.08 ± 0.73 (22.67–35.25)	.47
Home BP, mmHg			
Home MAP	96.17 ± 2.14 (75.06–121.50)	97.34 ± 2.21 (82.39–110.94)	.66
Home SBP	131.04 ± 2.63 (105.17–162.33)	135.03 ± 2.75 (115.17–154.50)	.29
Home DBP	78.77 ± 1.90 (60–103)	78.68 ± 2.25 (62.83–91.17)	.98
Study BP, mmHg			
Study MAP	103.73 ± 2.58 (75.33–139.89)	106.90 ± 2.57 (79.83–126.56)	.42
Study SBP	139.39 ± 3.18 (109.33–189.67)	142.48 ± 3.44 (112.5–166.33)	.53
Study DBP	85.90 ± 2.37 (58.33–115)	89.11 ± 2.32 (63.50–108.00)	.37
TG, mmol/L	1.38 ± 0.11 (0.36–3.76)	1.15 ± 0.07 (0.71–1.88)	.15
TC, mmol/L	5.36 ± 0.16 (3.30–7.69)	5.60 ± 0.27 (3.90–7.79)	.41
LDL-C, mmol/L	3.61 ± 0.14 (1.73–5.69)	3.82 ± 0.27 (2.09–6.53)	.45
HDL-C, mmol/L	1.49 ± 0.06 (0.92–2.21)	1.58 ± 0.09 (1.08–2.22)	.35
LDL-C/HDL-C	2.57 ± 0.14 (1.05–3.97)	2.57 ± 0.23 (1.07–4.35)	.98
TC/HDL-C	3.78 ± 0.17 (2.01–5.49)	3.72 ± 0.25 (2.03–5.77)	.79
HbA1c, mmol/mol	36.26 ± 0.63 (28–43)	35.79 ± 0.83 (26–40)	.67
D-dimer, µg/L	416.43 ± 59.05 (45–1616)	395.15 ± 52.52 (45–955)	.98
IL-6, pg/ml	0.47 ± 0.04 (0.15–1.25)	0.55 ± 0.08 (0.21–1.85)	.37

Note. Values are means ± *SEM* (range); BMI = body mass index; BP = blood pressure; MAP = mean arterial blood pressure; DBP = diastolic blood pressure; SBP = systolic blood pressure; TG = triglycerides; TC = total cholesterol; LDL-C = low-density lipoprotein cholesterol; HDL-C = high-density lipoprotein cholesterol; HbA1c = hemoglobin A1c; IL-6 = interleukin-6.

Table 3.A 2: Blood Lipid Reactivity in Hypertensive and Normotensive Participants

	Hypertensives (*n* = 28)	Normotensives (*n* = 28)	*p*
TC/HDL-C			
−1 min	4.14 ± 0.19 (2.46–6.29)	3.54 ± 0.17 (2.13–5.65)	.023*
+1 min	4.24 ± 0.20 (2.45–6.28)	3.54 ± 0.17 (2.15–5.51)	.009**
+10 min	4.24 ± 0.20 (2.44–6.52)	3.54 ± 0.17(2.16–5.62)	.010*
+20 min	4.22 ± 0.20 (2.43–6.26)	3.53 ± 0.17 (2.17–5.53)	.010*
+60 min	4.20 ± 0.20 (2.42–6.14)	3.51 ± 0.17 (2.12–5.58)	.011*
TG, mmol/L			
−1 min	1.66 ±0.14 (0.61–3.61)	1.38 ± 0.11 (0.35–2.87)	.121
+1 min	1.61 ± 0.13 (0.62–3.44)	1.24 ± 0.09 (0.37–2.21)	.022*
+10 min	1.60 ± 0.13 (0.67–3.44)	1.24 ± 0.10 (0.39–2.30)	.027*
+20 min	1.63 ± 0.13 (0.68–3.22)	1.23 ± 0.09 (0.39–2.30)	.014*
+60 min	1.65 ± 0.13 (0.71–3.41)	1.24 ± 0.09 (0.39–2.29)	.013*
TC, mmol/L			
−1 min	4.97 ± 0.17 (3.16–6.53)	4.63 ± 0.18 (2.69–7.01)	.18
+1 min	4.95 ± 0.18 (3.29–6.60)	4.66 ± 0.18 (2.80–7.05)	.25
+10 min	4.99 ± 0.19 (3.20–7.13)	4.76 ± 0.19 (2.87–7.21)	.39
+20 min	5.03 ± 0.19 (3.26–7.16)	4.66 ± 0.18 (2.77–7.15)	.16
+60 min	5.03 ± 0.19 (3.20–6.93)	4.73 ± 0.19 (2.79–7.41)	.26
HDL-C, mmol/L			
−1 min	1.25 ± 0.06 (0.87–1.93)	1.35 ± 0.05 (0.86–1.83)	.20
+1 min	1.21 ± 0.05 (0.80–1.96)	1.35 ± 0.05 (0.83–1.82)	.06
+10 min	1.23 ± 0.07 (0.67–2.02)	1.38 ± 0.05 (0.86–1.78)	.09
+20 min	1.24 ± 0.06 (0.87–1.97)	1.36 ± 0.05(0.83–1.79)	.14
+60 min	1.24 ± 0.06 (0.86–2.01)	1.38 ± 0.05 (0.86–1.88)	.08
LDL-C, mmol/L			
−1 min	3.31 ± 0.15 (1.63–4.51)	2.95 ± 0.16 (1.36–5.37)	.11
+1 min	3.30 ± 0.16 (1.71–4.61)	2.92 ± 0.18 (0.57–5.48)	.13
+10 min	3.31 ± 0.16 (1.67–5.00)	3.06 ± 0.17 (1.43–5.59)	.27
+20 min	3.36 ± 0.16 (1.73–4.99)	3.00 ± 0.16 (1.39–5.58)	.13
+60 min	3.38 ± 0.16 (1.72–5.04)	3.06 ± 0.17 (1.42–5.64)	.18

Note. Values are means ± SEM (range); TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; TG = triglycerides; LDL-C = low-density lipoprotein cholesterol. * $p < .005$; ** $p < .01$.

Empirical Study 4

Lower Diurnal HPA-Axis Activity in Male Hypertensive and Coronary Heart Disease Patients Predicts Future CHD Risk

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Abstract

Background. Coronary heart disease (CHD) and its major risk factor hypertension have both been associated with altered activity of the hypothalamus-pituitary-adrenal (HPA)-axis but the biological mechanisms underlying prospective associations with adverse disease outcomes are unclear. We investigated diurnal HPA-axis activity in CHD-patients, hypertensive (HT) and healthy normotensive men (NT) and tested for prospective associations with biological CHD risk factors.

Methods. Eighty-three male CHD-patients, 54 HT and 54 NT men repeatedly measured salivary cortisol over 2 consecutive days. Prospective CHD risk was assessed by changes between baseline and follow-up in the prothrombotic factors D-dimer and fibrinogen, the pro-inflammatory measures interleukin (IL)-6, tumor necrosis factor alpha (TNF-α), and acute phase protein C-reactive protein (CRP), as well as blood lipids in terms of total cholesterol (tChol)/high-density-lipoprotein cholesterol (HDL)-ratio[2]. We aggregated coagulation and inflammatory measures to respective indices.

Results. The groups differed in repeated daytime cortisol (dayCort) secretion ($p = .005$, $\eta^2_p = .03$, $f = 0.18$) and cortisol awakening response (CAR; $p = .006$, $\eta^2_p = .03$, $f = 0.18$), with similarly lower overall dayCort and CAR in CHD-patients and HT, as compared to NT. The groups differed further in cortisol at awakening ($p = .015$, $\eta^2_p = .04$, $f = 0.20$) with highest levels in HT ($ps \leq .050$), and in diurnal slope between waking and evening cortisol ($p = .033$, $\eta^2_p = .04$, $f = 0.20$) with steepest slopes in HT ($ps \leq .039$), although in part not independent of confounders. Lower aggregated dayCort and CAR in terms of area under the curve (AUC) independently predicted increases in future overall CHD risk ($AUC_{dayCort}$: $p = .021$, $\eta^2_p = .10$, f

[2] The abbreviation tChol/HDL (and not TC/HDL-C as in the rest of the thesis) has been used in this chapter as well as in the publication to avoid confusion with the abbreviations for cortisol.

= 0.33; AUC_{CAR}: p = .028, η^2_p = .09, f = 0.31) 3.00 ± 0.06 (*SEM*) years later, with risk prediction most pronounced in fibrinogen ($AUC_{dayCort}$: p = .017, ΔR^2 = 0.12; AUC_{CAR}: p = .082).

Conclusion. We found evidence for an HPA-axis hypoactivity in CHD and HT with lower diurnal HPA-axis activity predicting increases in cardiovascular risk as evidenced by increases in circulating levels of biomarkers of atherothrombotic risk. Down-regulation of basal HPA-axis activity may contribute to the pathogenesis of atherosclerosis and thrombosis in CHD via effects on coagulation.

Keywords: HPA-axis, cortisol, coronary heart disease, hypertension, coagulation

Introduction

Coronary heart disease (CHD) ranks among the leading causes of death in western countries (Virani et al., 2021). A major risk factor for CHD is hypertension (HT), a chronic elevation of blood pressure (BP; Whelton et al., 2018). Most individuals with hypertension are diagnosed with "essential hypertension" with unknown medical cause (Carretero & Oparil, 2000; Messerli et al., 2007). HT and CHD have both been associated with alterations in diurnal activity of the hypothalamus-pituitary-adrenal (HPA)-axis (see below, e.g., Molloy et al., 2008; Wirtz et al., 2007), but its relevance with respect to mechanisms of disease progression is not fully understood.

The HPA-axis end-product cortisol is important for the integrity of central nervous system function and for maintenance of cardiovascular, metabolic, and immune homeostasis (Sapolsky et al., 2000). The basal HPA-axis activity follows a diurnal rhythm characterized by the cortisol awakening response (CAR), a sharp rise by a about 50% to over 100% within the first 30-to-45 min after awakening (Clow et al., 2004; Fries et al., 2009; Pruessner et al., 1997; Stalder et al., 2016). This morning peak is followed by a circadian decline with a steady decrease of cortisol throughout the day and minimum levels at midnight (Ross et al., 2014; Stone et al., 2001). Dysregulated circadian cortisol profiles often comprise either high cortisol levels throughout the day and a flattened diurnal rhythm (HPA-axis hyperactivity), or lower overall cortisol secretion with a flatter diurnal slope and lower morning cortisol levels (HPA-axis hypoactivity; for review see Collomp et al., 2016).

A variety of studies *cross-sectionally* assessed basal HPA-axis activity in HT and heart patients or individuals with CHD-symptoms, respectively. Basal HPA-axis activity was compared between individuals with *HT* and normotensives (i.e., a normal blood pressure, NT). Male and female HT who discontinued medication-intake had higher morning cortisol levels compared to NT (Kidambi et al., 2007), but not unequivocally (Wirtz et al., 2007). With respect

to the CAR, we previously found a blunted CAR in unmedicated HT (Wirtz et al., 2007) and also medicated hypertensive men and women showed lower aggregated total CAR compared to NT (Pulopulos et al., 2016). Similarly, in predominantly non-hypertensive male and female participants, higher BP related to a lower aggregated total CAR (Kuehl et al., 2015). Regarding diurnal cortisol profiles, we could not previously observe differences between medication-free HT and NT (Wirtz et al., 2007). Evening cortisol was higher in HT compared to NT (Kidambi et al., 2007). In *heart disease patients*, salivary waking or morning cortisol was not associated with CHD (or markers of subclinical CHD) in most studies (Bhattacharyya et al., 2008; Hajat et al., 2013; Nijm et al., 2007). Patients with cardiovascular disease (CVD) showed flatter CARs than non-CVD participants (Vreeburg et al., 2009) and hypertensive patients with acute coronary syndrome (ACS) had smaller aggregated CARs than normotensive patients (Molloy et al., 2008), but there are also contradicting findings (Bhattacharyya et al., 2008). With respect to salivary cortisol daytime levels, CHD-patients did not differ in cortisol slopes or total cortisol output over the day from participants without a CHD-diagnosis but symptoms (Bhattacharyya et al., 2008). Regarding *associations with markers of subclinical CHD*, salivary waking or morning cortisol was not associated with CHD (or markers of subclinical CHD) in most studies (Bhattacharyya et al., 2008; Hajat et al., 2013; Nijm et al., 2007). Various measures reflecting a flatter CAR were associated with higher values of intima-media-thickness (Hurwitz Eller et al., 2001) and ankle-brachial-index (ABI; Hajat et al., 2013) in women and coronary artery calcification (CAC) in men and women (Hajat et al., 2013), but not unequivocally (see also Whitehead et al., 2007). With respect to diurnal cortisol, a flatter slope over the day was associated with the recurrence of cardiac symptoms in ACS-patients (Molloy et al., 2008) and with higher CAC in population-based studies (Matthews et al., 2006). Also, higher total cortisol output was related to more carotid plaques (Dekker et al., 2008). However, there are also diverging diurnal cortisol findings (Dekker et al., 2008; Hajat et al.,

2013; Matthews et al., 2006; Whitehead et al., 2007). Bedtime cortisol levels were higher in ACS-patients compared to healthy controls (Nijm et al., 2007). Taken together, the above-described cross-sectional findings point to a reduced CAR, flatter slopes over the day, and higher cortisol evening levels in hypertensive individuals as well as in heart patients. However, to the best of our knowledge, it has not yet been investigated whether HT differ from CHD-patients in their basal HPA-axis activity or whether the separately observed dysregulations are comparable in both groups.

Prospective evidence is emerging that a dysregulated basal HPA-axis activity relates to adverse heart disease outcomes, but the underlying biological mechanisms with respect to disease progression are unclear. Lower waking cortisol in patients undergoing coronary artery bypass graft (CABG) surgery predicted cardiac events and death about 3 years later (Ronaldson et al., 2015). Moreover, a flatter diurnal cortisol decline predicted cardiac events and death in CABG-patients (Ronaldson et al., 2015), and CVD-related mortality in Whitehall-II participants (Kumari et al., 2011). Finally, higher bedtime or evening cortisol levels predicted mortality risk (Hammer et al., 2016; Kumari et al., 2011), adverse clinical outcomes (Ronaldson et al., 2015), and incidence of fatal CHD (Groffen et al., 2015), predominantly in heart patients. The mechanisms underlying these prospective associations have rarely been investigated. So far, only two non-patient studies suggest prospective associations that point to the process of atherosclerosis. In policemen, a flatter aggregated CAR predicted a larger 7-year mean decline in brachial artery flow-mediated dilation indicative of endothelial dysfunction (Violanti et al., 2018). Moreover, in a population-based study, healthy women with flatter diurnal cortisol slopes and higher bedtime levels showed greater progression of aortic stiffness 5 years later (Ikeda et al., 2021).

Independent biological CHD risk factors that underly the process of atherosclerosis and thrombosis include markers of coagulation, inflammation, and hyperlipidemia (Danesh et al.,

2008; Ridker et al., 2001; Simes et al., 2018; Tuomisto et al., 2006). To date, despite evidence for cross-sectional associations (DeSantis et al., 2012; Lippi et al., 2008; Nijm et al., 2007; Piazza et al., 2018; Rosmond & Björntorp, 2000), prospective evidence addressing associations between basal HPA-axis activity and independent biological CHD risk factors is lacking so far, not only in healthy participants, but also in hypertension or CHD. A better understanding of the biological mechanisms underlying disease progression may have implications for longer-term therapy in vulnerable populations such as HT and heart patients.

To close the above described gaps in current knowledge, the first objective of our study was to cross-sectionally compare diurnal HPA-axis activity between CHD-patients, HT, and controls with neither HT or CHD (NT). We repeatedly measured salivary cortisol over 2 consecutive days and hypothesized a blunted CAR, flatter diurnal slopes, and higher evening cortisol levels in both, CHD-patients and HT, as compared to NT. Second, to obtain new mechanistic insights with respect to the clinical relevance of basal HPA-axis activity, we prospectively investigated whether cortisol would predict changes in biological CHD risk factors including markers of coagulation, inflammation, and hyperlipidemia over a mean follow-up of 3 years.

Materials and Methods

Study Participants

The current investigation is part of a study program assessing psychoneurobiological mechanisms in essential hypertension and CHD (Auer et al., 2022; Degroote et al., 2021; Zuccarella-Hackl, von Känel, Thomas, Hauser, et al., 2016; Zuccarella-Hackl, von Känel, Thomas, Kuebler, et al., 2016). It was approved by the ethics committee of the Canton of Bern, Switzerland and the study protocol is in accordance with the Declaration of Helsinki. All

participants provided written informed consent and were financially compensated for each assessment with CHF 20.

We restricted our study sample to male individuals given the prevalence of cardiovascular diseases at earlier age (e.g., Mosca et al., 2011; Roth et al., 2020), given sex differences in HPA-axis activity (e.g., Fries et al., 2009), and given the differences between men and women regarding the associations of diurnal cortisol secretion and CHD-symptoms (e.g., Hurwitz Eller et al., 2001). We recruited male participants with either a diagnosis of CHD, apparently healthy individuals with essential hypertension without CHD, or healthy normotensive controls and asked them to provide saliva samples for the assessment of diurnal cortisol profiles. The final cross-sectional sample comprised 191 participants, with 83 CHD-patients, 54 HT (42 medication-free, 12 medicated) as well as 54 NT. All study participants were invited for a follow-up assessment, with 106 subjects (NT: n = 32, HT: n = 31, CHD-patients: n = 43) completing both assessments (after an average of 3 years; 3.00 ± 0.06 *SEM*). More information is detailed in the Supplemental Material.

Reasons for drop-out at follow-up, included could not be reached by phone (n = 9), lack of time (n = 16), no interest (n = 12), excessive demands (n = 7), severe illness (n = 8), meanwhile living abroad (n = 2), discontent with the study management (n = 1), deceased (n = 2), or no specific reason given (n = 26). Further, two participants had to be excluded because of acute infection on the follow-up study day. Notably, due to organizational reasons (relocation of the working group from Bern to Konstanz) the number of available follow-up time-slots per month was substantially reduced compared to the baseline assessment, resulting in a prolonged follow-up time and potentially responsible for the attrition. Attrition was comparable across the study groups (drop-out rate: NT = 40.7%; HT = 42.6%; CHD = 48.2%; $Chi^2(2) = .85$, $p = .66$).

Recruitment and General Inclusion Criteria.

CHD-Patients. We included patients with stable CHD who had been discharged from the Cardiac Prevention and Rehabilitation Clinic of the Bern University Hospital at least 6 months ago. We asked those patients of the Cardiac Prevention and Rehabilitation Clinic of the Bern University Hospital who volunteered to be contacted for the purpose of scientific studies. All patients were diagnosed with CHD based on coronary angiography and we provide information regarding myocardial infarction (MI), left ventricular ejection fraction (LVEF) < 40%, and CABG in Table 4.1. All patients were under medication based on current guidelines and in stable compensated cardiac conditions (Knuuti et al., 2019).

Essential Hypertension and Normotension. We recruited apparently healthy, nonsmoking hypertensive and normotensive men of comparable age by aid of the Swiss Red Cross of Bern. Members of our study team accompanied the mobile blood-donation unit that routinely records BP ranges before blood donation. Interested blood donors were given written study information asking for the following inclusion criteria: age between 18–80 years; BP either in the hypertensive or in the normotensive range (see below); smoking less than five cigarettes per day; and no alcohol or illicit drug abuse. We accepted intake of antihypertensive medication in a small proportion to increase sample size of hypertensive individuals. Apart from hypertension-related criteria, NT and HT were required to meet the same inclusion and exclusion criteria (alcohol and illicit drug abuse, strenuous exercise, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, human immunodeficiency virus, cancer, major psychiatric disorders, neurological diseases, and current infectious diseases) as verified by telephone interview using an extensive health questionnaire (Zuccarella-Hackl, von Känel, Thomas, Hauser, et al., 2016; Zuccarella-Hackl, von Känel, Thomas, Kuebler, et al., 2016). Four eligible participants (NT: n = 2, HT: n = 2) who reported regular medication intake stopped medication 1 week prior to participating in the

study. To exclude potential cases with secondary hypertension, eligible HT provided blood samples for the routine assessment of serum creatinine, calcium, sodium, and potassium (Unger et al., 2020). No eligible HT was diagnosed with secondary hypertension. We measured HbA1c in all participants. Furthermore, we recruited 12 participants previously diagnosed as hypertensives, who were under antihypertensive medication.

Classification of Essential Hypertension and Normotension. Classification of essential hypertension and normotension of the unmedicated participants was based on a two-step assessment procedure, while medicated hypertensive individuals and CHD-patients were assigned a priori to the study groups.

(1) Home Blood Pressure Measurement. Following written instructions, interested blood donors were asked to measure BP on 3 days at home using an upper arm digital blood pressure monitor (Omron M6; Omron-Healthcare-Europe B.V., Hoofdorp, Netherlands). Home BP was to be measured twice a day (once in the morning and in the evening) in a seated position after a 15-min rest. Participants were recruited as hypertensive if the average home systolic BP (SBP) was ≥ 135 mmHg and/or the average home diastolic BP (DBP) was ≥ 85 mmHg according to recommendations for home BP measurements (Williams et al., 2018). Correspondingly, participants were recruited as normotensive if the average home SBP was < 135 mmHg and the average home DBP was < 85 mmHg. Rendering a minimum of three and a maximum of six measurements for each participant, we computed the average home BP.

(2) Study Blood Pressure Measurement. To verify the home-measurement based preliminary classification of each medication-free participant as hypertensive or normotensive, trained personnel performed three additional BP measurements during the study session in a seated position after a 15-min rest by means of sphygmomanometry (Omron M6; Omron-Healthcare-Europe B.V., Hoofdorp, Netherlands). We applied the regular World Health Organization/International Society of Hypertension definition of hypertension and classified

medication-free participants as hypertensive if their average study SBP was ≥ 140mmHg and/or their average study DBP was ≥ 90mmHg (Chalmers et al., 1999). Medication-free participants were classified as normotensive if their average study SBP was < 140mmHg and their average study DBP was < 90mmHg. The final group assignment of medication-free participants was based on congruent home and study BP classification.

Design and Procedure

In anticipation of the experimental session, all participants consumed a semi-standardized breakfast following written instructions and abstained from caffeine and alcohol consumption 24 hr prior to their arrival at the lab at 8:00h. Questionnaires were administered, and participants´ height and weight were measured. Participants received material and written instructions for saliva collection at home, before resting study BP was assessed.

To assess longitudinal changes in CHD risk factors, blood samples were collected at 11:30h, i.e., after a fasting for 3.5 hr since arrival. All participants were invited for identical blood sampling procedures scheduled after a minimum of 1.5 years later (mean ± *SEM* = 3.00 ± 0.06).

Cortisol Sampling Protocol

Study participants were asked to obtain saliva samples on 2 consecutive workdays using salivette collection devices (Sarstedt, Rommelsdorf, Germany). To assess the CAR, five saliva samples were collected immediately after awakening and 15, 30, 45, and 60 min (S1-to-S5) thereafter. Further samples were taken at 16:00h and 22:00h (S6-to-S7). Participants were free to wake up in accordance with their normal schedule, but at the latest by 8:00h. They had to remain lying in bed for the first 15 min, and to abstain from breakfast during the first 30 min, i.e., until after collection of the +30 min after awakening salivette. For the breakfast that followed, participants were asked to avoid coffee or juicy drinks. Moreover, participants were

instructed not to brush their teeth during the 1st hr after awakening. They were also told to clean their mouth with water before each saliva collection. In addition, participants were instructed to complete a diary during the sampling period, assessing bed- and wake-up times as well as the accurate sampling times. In addition to self-reports, we used electronic monitoring devices (MEMS Track Cap, Aardex, Switzerland).

A total of 123 participants provided accurate cortisol samples for both consecutive sampling days, whereas 15 participants provided accurate cortisol samples but for 2 non-consecutive days. Furthermore, CAR data of 53 participants were accurate for only 1 sampling day because of incomplete (n = 33) or inaccurate sampling (n = 20) of the other day. See Supplemental Material for more details.

Biochemical Analyses

Cortisol. Participants were instructed to store their saliva samples in the refrigerator until sampling completion and to then send the collected samples to our laboratory as fast as possible. We stored saliva samples until study completion at −20 °C. Biochemical analyses of cortisol [nmol/L] were performed with a competitive time-resolved fluorescence immunoassay (DELFIA; Dressendörfer et al., 1992) in the Biochemical Laboratory of the University of Trier. Intra- (4.0–6.7%) and inter-assay (7.1–9.0%) coefficients of variation were ≤ 9.0%.

Prospective CHD Risk Assessment. We assessed prospective CHD risk by measuring changes between baseline and follow-up assessment of the following biological risk factors: (1) the prothrombotic factors D-dimer [µg/L] and fibrinogen [g/L], the (2) pro-inflammatory measures interleukin (IL)-6 [pg/ml], tumor necrosis factor alpha (TNF-α) [pg/ml], and the acute phase protein C-reactive protein (CRP) [µg/ml], and (3) blood lipid profiles in terms of total cholesterol (tChol)/high-density lipoprotein cholesterol (HDL)-ratio. Fibrinogen and D-dimer were analyzed at the Center for Laboratory Medicine of the Bern University Hospital

(Inselgruppe AG, Bern) applying standard quality procedures following the Clauss method (Clauss, 1957; fibrinogen) and a particle-enhanced immunoturbidimetric assay (INNOVANCE® D-Dimer, Siemens Healthcare GmbH, Erlangen, Germany), respectively. Blood lipids were also analyzed in Bern using in vitro assays (enzymatic colorimetric, Roche, Mannheim, Germany). IL-6, TNF-α, and CRP were analyzed in the biochemical laboratory of the Biological Work and Health Psychology group at the University of Konstanz. Cytokines were determined with a high sensitivity chemiluminescence sandwich immunoassay (Meso Scale Discovery, Rockville, USA), while CRP was determined using a high-sensitive enzyme immunoassay (ELISA, IBL Hamburg, Germany). For more details see Supplemental Methods.

HbA1c. HbA1c analyses were performed with in vitro assays for the quantitative determination of HbA1c IFCC [mmol/mol] in whole blood (Tina-quant®, Roche, Mannheim, Germany; see Supplemental Methods).

Statistical Analyses

Statistical analyses were performed using SPSS (Version26.0) statistical software packages for MacIntosh (IBM SPSS Statistics, Chicago IL, USA). All tests were two-tailed with level of significance set at $p < .05$. No outliers were excluded.

We a priori calculated power-analyses using G∗Power3.1. Following our previous findings, we expected an effect size of $f = 0.35$ with respect to group differences between HT and NT in CAR (Wirtz et al., 2007). Based on our hypotheses we expected comparably smaller differences between CHD-patients and HT. To allow to detect small effects of $f = 0.10$ in a 3(groups)-by-5(measurement points) repeated measurement ANOVA with a power of 90% and an observed average correlation of the repeated measures of $r = .54$ in cross-sectional analyses, the required total sample size is $N = 180$.

A posteriori, we determined *f* from partial η^2 (η^2_p) values using G*Power3.1. Effect size parameters *f* and R^2 changes are reported where appropriate (effect size conventions; small: $f = 0.10$, $\Delta R^2 = .02$; medium: $f = 0.25$, $\Delta R^2 = .13$; large: $f = 0.40$, $\Delta R^2 = .26$; Cohen, 1988).

For all participants, we calculated mean arterial BP (MAP) based on the three BP study measurements by the formula MAP = (2/3 * mean study DBP) + (1/3 * mean study SBP) and body mass index (BMI) by the formula BMI = kg/m^2.

For data and measures relating to cortisol, we calculated mean values of the 2 sampling days. To aggregate diurnal cortisol profiles for prospective analyses, we calculated mean total diurnal cortisol released during sampling days computed as area under the curve with respect to ground ($AUC_{dayCort}$: S1-to-S7). Total CAR´s were calculated accordingly (AUC_{CAR}: S1-to-S5; Pruessner et al., 2003). Diurnal cortisol slopes were estimated following previous recommendations with one formula anchoring cortisol levels at awakening ($Slope_{Awake}$) and the other anchoring the individual morning peak ($Slope_{Peak}$; Adam et al., 2017). Fibrinogen, D-dimer, tChol/HDL-ratio, IL-6, CRP, and TNF-α changes from baseline to follow-up assessment were computed by subtracting baseline values from follow-up values, with higher change values indicating increases in the respective parameters over time, i.e., over the follow-up period. Building on previous methods (e.g., Eapen et al., 2013; Piazza et al., 2018; von Känel & Orth-Gomér, 2008), we computed an aggregated coagulation index by averaging z-transformed change values of D-dimer and fibrinogen. For an aggregated inflammatory index, we accordingly averaged z-transformed change values of IL-6, CRP, and TNF-α.

All data were tested for normal distribution and homogeneity of variance using Kolmogorov-Smirnov and Levene's tests prior to statistical analyses. All measures showing a skewed distribution (see Supplemental Material) were log-transformed. While log-transformed data were used for modeling and testing, we depict untransformed data in Tables 4.1 and 4.2,

in Figure 4.1 and in Supplemental Tables S4.1 and S4.2 for reasons of clarity. Figure 4.2 depicts residuals of the dependent and independent variables adjusted for the full set of covariates.

To compute group differences in subject characteristics (Table 4.1) we used univariate ANOVAs. Cross-sectionally, to analyze whether groups differed in total diurnal cortisol secretion, we calculated repeated measures ANOVAs and ANCOVAs with repeated assessment of cortisol (S1–S7), as the dependent variable and group as the independent variable. Post hoc testing comprised separate testing for differences between the three groups in the CAR (S1–S5), cortisol at awakening (S1), diurnal cortisol slopes (awake-to-last, peak-to-last), as well as evening cortisol (S7). We controlled for possible confounding effects of awakening time, sleep duration the night before saliva sampling, and medication intake in HT, in addition to age and BMI in repeated measures and univariate ANCOVAs (Stalder et al., 2016). Further post hoc testing comprised repetition of the previous cortisol analyses but with comparisons of two subject groups instead of three (i.e., HT-vs.-NT, CHD-vs.-NT, and CHD-vs.-HT). We applied Huynh-Feldt correction for repeated measures.

We calculated prospective analyses to shed light on the potential clinical relevance of basal HPA-axis activity. We tested whether diurnal cortisol parameters would predict future changes in CHD risk factors. We calculated multivariate analyses of covariance (MANCOVA) with prospective changes in blood lipids (tChol/HDL-ratio change), as well as the coagulation and inflammatory indexes as dependent variables. As the main independent variable of interest, we entered aggregated cortisol daytime levels ($AUC_{dayCort}$). To avoid model overfitting given the reduced sample size of $N = 106$ in our prospective analyses allowing for a maximum of 11 covariates simultaneously (Babyak, 2004), covariates were entered setwise as follows: the default set of covariates comprises age at baseline, time between baseline and follow-up assessments, and medication intake in normotensive and hypertensive individuals at follow-up (model 1). Sleep duration and wake-up time (model 2), study group (model 3), and BMI at

baseline in addition to prospective changes in BMI and MAP (model 4) were added successively as covariates to each previous model in complementary analyses (see Table 4.3). We post hoc tested significant multivariate effects of aggregated cortisol daytime levels on future CHD risk by repeating the above described MANCOVA procedure while entering the separate parts of daytime cortisol secretion, i.e., CAR, diurnal slopes, and evening cortisol, as independent variable. Post hoc testing of significant between-subject effects of cortisol parameters on any of the three dependent variables comprised linear regression analyses including changes where appropriate (e.g., D-dimer and fibrinogen change levels if the coagulation index was significant).

Results

Group Characteristics

Table 4.1 provides demographic and medical characteristics of CHD-patients, as well as hypertensive and normotensive participants. The three study groups differed in terms of age and BMI: CHD-patients had the highest average age ($p \leq .001$; mean ± *SEM*: CHD: 65.02 ± 0.99; HT: 52.74 ± 1.57; NT: 50.80 ± 1.63), whereas HT had a higher BMI than the other groups ($p < .001$; mean ± *SEM*: CHD: 27.85 ± 0.43; HT: 28.51 ± 0.52; NT: 25.27 ± 0.33). As expected, HT showed the highest study values in systolic BP, diastolic BP, and MAP compared with NT and CHD-patients ($p < .001$). HT showed the highest CRP ($p < .004$) and tChol/HDL-ratios ($p < .001$), while CHD-patients had highest fibrinogen and HbA1c levels ($ps \leq .010$). On average, HT had serum levels of creatinine, calcium, sodium, and potassium in the normal reference range, thus supporting a diagnosis of essential hypertension. No participant had a diagnosis of a disease affecting the basal activity of the HPA-axis, such as adrenal insufficiency. In addition, all patients were under medication but no participant was treated with glucocorticoid substitution therapy (see Supplemental Material).

Table 4. 1: Group Characteristics, Diurnal Cortisol, and Intermediate Biological CHD Risk Factors at Baseline

	CHD *n* = 83	HT *n* = 54	NT *n* = 54	*p*			
				NT, HT, vs. CHD	NT vs. HT	NT vs. CHD	HT vs. CHD
Age, years	65.02 ± 0.99 (44–85)	52.74 ± 1.57 (21–74)	50.80 ± 1.63 (25–78)	**<.001**	.39	**<.001**	**<.001**
BMI, kg/m^2	27.85 ± 0.43 (21.97–46.44)	28.51 ± 0.52 (20.35–38.86)	25.27 ± 0.33 (19.78–30.85)	**<.001**	**<.001**	**<.001**	.31
Study BP, mmHg							
Study SBP	141.57 ± 1.64 (111.67–187.33)	151.96 ± 1.86 (120.67–189.67)	127.09 ± 1.17 (109.33–139.67)	**<.001**	**<.001**	**<.001**	**<.001**
Study DBP	81.20 ± 1.09 (62.33–105.00)	93.99 ± 1.31 (72.67–115.00)	78.28 ± 1.03 (58.33–89.50)	**<.001**	**<.001**	.07	**<.001**
Study MAP	101.33 ± 1.11 (81.89–132.00)	113.31 ± 1.41 (88.67–139.89)	94.55 ± 1.01 (75.33–104.83)	**<.001**	**<.001**	**<.001**	**<.001**
Home BP, mmHg							
Home SBP		143.52 ± 1.28 (119.17–162.33) *n* = 52	122.76 ± 0.86 (105.17–134.60)		**< .001**		
Home DBP		85.67 ± 1.02 (68.33–103.00) *n* = 52	72.23 ± 0.80 (60.00–82.33)		**< .001**		
Medication*	*n* = 83	*n* = 12					
LVEF ≤ 40%, %	11 (13.3) *n* = 81	-	-				
MI, %	47 (56.6) *n* = 81	-	-				
CABG, %	23 (27.7) *n* = 81	-	-				
Smoking, %	5 (6.0)	-	-				
HbA1c, mmol/mol	41.49 ± 0.77 (33–72) *n* = 80	36.74 ± 0.52 (26–43) *n* = 53	36.29 ± 0.49 (26–42) *n* = 51	**<.001**	.58	**<.001**	**<.001**
Creatinine, µmol/L		80.76 ± 1.41 (64–103)					
Sodium, mmol/L		140.32 ± 0.30 (135–145) *n* = 47					
Calcium, mmol/L		2.37 ± 0.01 (2.11–2.58) *n* = 46					
Potassium, mmol/L		4.12 ± 0.04 (3.70–4.90) *n* = 47					
Cortisol							
Awakening, nmol/L	4.41 ± 0.29 (0.35–18.00)	5.38 ± 0.4 (0.68–14.83)	3.94 ± 0.39 (0.02–15.34)	**.015**	**.006**	.18	.05
16:00h, nmol/L	1.82 ± 0.14 (0.06–7.38)	1.77 ± 0.12 (0.14–4.29)	1.8 ± 0.16 (0.31–5.15)	.87	.97	.65	.68
22:00h, nmol/L	0.85 ± 0.07 (0.09–3.14)	0.75 ± 0.12 (0.04–6.10)	0.82 ± 0.10 (0.10–3.77)	.23	.49	.35	.08
$Slope_{Awake}$	−0.23 ± 0.02 (−1.00–0.18)	−0.29 ± 0.03 (−0.87–0.17)	−0.20 ± 0.03 (−0.96–0.17)	**.033**	**.021**	.44	**.039**
$Slope_{Peak}$	−0.46 ± 0.03 (−1.13–0.18)	−0.50 ± 0.03 (−1.11–0.18)	−0.49 ± 0.05 (−1.63–0.04)	.66	.89	.52	.37
M wake-up time, h	6:16 ± 0:05 (3:55–8:00)	6:05 ± 0:06 (4:20–7:27)	5:55 ± 0:06 (3:27–7:07)	**.040**	.27	**.014**	.18
M sleep duration, h	7.51 ± 0.10 (4.92–9.95)	7.28 ± 0.09 (5.94–9.00)	7.04 ± 0.10 (4.94–8.92)	**.004**	.07	**.002**	.15
Coagulation							
Fibrinogen, g/L	2.85 ± 0.06	2.62 ± 0.06	2.6 ± 0.08	**.010**	.73	**.009**	**.013**

	CHD *n* = 83	HT *n* = 54	NT *n* = 54	*p*			
	(1.65–4.46)	(1.47–3.72)	(1.57–4.25)				
D-dimer, µg/L	622.64 ± 92.37 (45–5177)	474.04 ± 30.41 (155–1047)	513.46 ± 47.36 (45–1616)	.94	.89	.85	.74
Inflammation							
IL-6, pg/ml	0.56 ± 0.04 (0.03–1.57) *n* = 82	0.57 ± 0.04 (0.16–1.48) *n* = 53	0.50 ± 0.07 (0.22–3.61) *n* = 53	.18	**.047**	.19	.54
TNF-α, pg/ml	2.09 ± 0.09 (0.71–5.11) *n* = 82	1.84 ± 0.06 (0.72–3.28) *n* = 53	2.07 ± 0.10 (0.88–4.91) *n* = 53	.16	.07	.95	.09
CRP, µg/ml	2.15 ± 0.22 (0.07–11.55) *n* = 78	2.83 ± 0.26 (0.64–8.65) *n* = 50	2.06 ± 0.30 (0.11–9.59) *n* = 40	**<.004**	**<.001**	.63	**<.002**
tChol/HDL	3.09 ± 0.08 (1.73–5.86) *n* = 80	3.99 ± 0.13 (2.38–6.41)	3.66 ± 0.13 (2.03–5.72) *n* = 51	**<.001**	.06	**<.001**	**<.001**

Note. Values are *M* ± *SEM*. CHD = CHD-patients; HT = hypertensive individuals; NT = normotensive individuals; BMI = body mass index; CABG = coronary artery bypass graft surgery; DBP = diastolic blood pressure; LVEF = left ventricular ejection fraction; MAP = mean arterial blood pressure; MI = myocardial infarction; SBP = systolic blood pressure; $Slope_{Awake}$ = slope anchored to awakening; $Slope_{Peak}$ = slope anchored to peak, *See Supplemental Table S4.1. Statistically significant results are highlighted in bold.

Diurnal HPA-Axis Activity

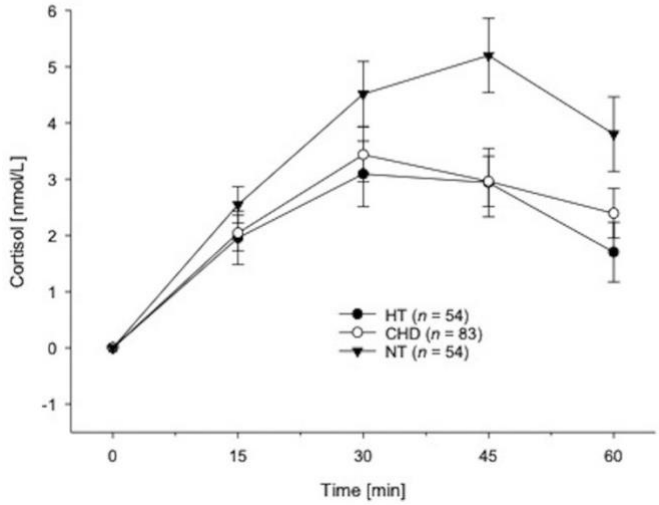
Repeated measures AN(C)OVAs with cortisol as repeated dependent variable (S1–S7) revealed that the three groups significantly differed in their total diurnal HPA-axis activity (interaction group-by-time: $F(8.16, 767.45) = 2.73$, $p = .005$, $\eta^2_p = .03$, $f = 0.18$; with covariates: $F(8.43, 771.31) = 1.95$, $p = .047$, $\eta^2_p = .02$, $f = 0.14$). As compared to normotensives, both HT and CHD-patients had lower cortisol concentrations during the day (HT-vs.-NT: interaction group-by-time: $F(3.83, 405.78) = 2.85$, $p = .026$, $\eta^2_p = .03$, $f = 0.18$); with covariates: $F(4.04, 407.75) = 3.31$, $p = .011$, $\eta^2_p = .03$, $f = 0.18$); CHD-vs.-NT: interaction group-by-time: $F(4.13, 556.91) = 2.75$, $p = .026$, $\eta^2_p = .02$, $f = 0.14$; with covariates: $F(4.25, 557.01) = 2.29$, $p = .055$, $\eta^2_p = .02$, $f = 0.14$). Moreover, CHD-patients had lower total diurnal HPA-axis activity as compared to HT, but not independent of covariates (interaction group-by-time: $F(3.97, 535.52) = 2.63$, $p = .034$, $\eta^2_p = .02$, $f = 0.14$); with covariates: $p = .54$).

Cortisol at Awakening and Cortisol Awakening Response. Post hoc testing of total diurnal HPA-axis activity comprised further analysis of cortisol levels within the 1st hr after awakening (see Figure 4.1).

Cortisol at Awakening. As depicted in Table 4.1, the three groups differed in their cortisol levels at awakening ($F(2, 188) = 4.28$, $p = .015$, $\eta^2_p = .04$, $f = 0.20$; with covariates: $F(2, 183) = 3.15$, $p = .045$, $\eta^2_p = .03$, $f = 0.18$). Cortisol awakening levels were highest in HT, in particular as compared to normotensives who showed lowest awakening levels (HT-vs.-NT: $F(1, 108) = 7.85$, $p = .006$, $\eta^2_p = .07$, $f = 0.27$; with covariates: $F(1, 101) = 8.38$, $p = .005$, $\eta^2_p = .08$, $f = 0.29$). Differences between HT and CHD-patients were of borderline significance (CHD-vs.-HT: $F(1, 137) = 3.91$, $p = .050$, $\eta^2_p = .03$, $f = 0.18$; with covariates: $p = .08$). However, despite higher cortisol awakening levels, CHD-patients did not significantly differ from normotensives (CHD-vs.-NT: $p = .18$, with covariates: $p = .20$).

Cortisol Awakening Response. Repeated measures AN(C)OVAs with cortisol (S1–S5) as repeated dependent variable showed significant CAR group differences (interaction group-by-time: $F(5.67, 532.65) = 3.10$, $p = .006$, $\eta^2_p = .03$, $f = 0.18$); with covariates: $F(5.82, 532.19) = 2.61$, $p = .018$, $\eta^2_p = .03$, $f = 0.18$). As compared to normotensives, HT and CHD-patients showed a lower CAR (HT-vs.-NT: interaction group-by-time: $F(2.61, 276.85) = 4.89$, $p = .004$, $\eta^2_p = .04$, $f = 0.20$; with covariates: $F(2.75, 277.73) = 4.59$, $p = .005$, $\eta^2_p = .04$, $f = 0.20$; CHD-vs.-NT: interaction group-by-time: $F(2.70, 365.06) = 4.34$, $p = .007$, $\eta^2_p = .03$, $f = 0.18$; with covariates: $F(2.80, 366.70) = 4.23$, $p = .007$, $\eta^2_p = .03$, $f = 0.18$). However, HT and CHD-patients did not differ in their CAR ($p = .80$; with covariates: $p = .59$).

Figure 4. 1: Cortisol Awakening Responses in the Three Study Groups



Note. Cortisol awakening responses in hypertensive individuals, CHD-patients and normotensive participants without CVD calculated in terms of repeated measures ANOVAs and depicted as absolute changes from cortisol awakening concentrations (mean ± *SEM*).

Diurnal Decline and Evening Cortisol. The groups differed in terms of diurnal cortisol decline from awakening to evening, but not independently of covariates ($Slope_{Awake}$: $F(2, 188) = 3.48$, $p = .033$, $\eta^2_p = .04$, $f = 0.20$; with covariates: $p = .11$). Diurnal decline from awake to evening was steepest in HT who differed from normotensives with flattest awakening levels (HT-vs.-NT: $F(1, 106) = 5.53$, $p = .021$, $\eta^2_p = .05$, $f = 0.23$; with covariates: $F(1, 101) = 4.29$, $p = .041$, $\eta^2_p = .04$, $f = 0.20$). HT and CHD-patients in terms of diurnal decline but not independently of covariates (HT-vs.-CHD: $F(1, 135) = 4.34$, $p = .039$, $\eta^2_p = .03$, $f = 0.18$: with covariates: $p = .09$), but CHD-patients did not significantly differ from normotensives (CHD-vs.-NT: $p = .44$; with covariates: $p = .67$).

In terms of evening cortisol, HT showed lower levels as compared to CHD-patients (HT-vs.-CHD: $p = .08$; with covariates: $p = .79$; HT-vs.-NT: $p = .49$; CHD-vs.-NT: $p = .35$; see Table 4.1).

Prediction of Future CHD Risk by Diurnal HPA-Axis Activity

Our main MANCOVA analysis revealed that higher daytime cortisol levels in terms of $AUC_{dayCort}$ significantly related to future overall CHD risk that comprised the dependent variables of the MANCOVA: tChol/HDL-ratio change, coagulation and inflammatory indices (MANCOVA multivariate effects: model 1: $F(3, 99) = 3.39$, $p = .021$, $\eta^2_p = .10$, $f = 0.33$, Wilk`s $\Lambda = .91$). Additional controlling for further covariates (models 2-to-4) did not alter the significance of this multivariate effect ($ps \leq .039$, see Table 4.3). $AUC_{dayCort}$ levels were significantly associated with the coagulation index (MANCOVA between-subject effects: model 1: $F(1, 101) = 7.56$, $p = .007$, $\eta^2_p = .07$; $f = 0.27$; models 2-to-4: ($ps \leq .007$) but not with the inflammatory index ($ps \geq .51$) or prospective changes in tChol/HDL-ratio ($ps \geq .85$). Further analysis revealed that $AUC_{dayCort}$ predicted greater increases from baseline to follow-up in fibrinogen (regression analyses: model 1: ß = −.23, $p = .017$, $\Delta R^2 = .12$; model 2-to-4: ßs ≥ −.26, $ps \leq .012$, $\Delta R^2 \geq .14$) but not D-dimer ($ps \geq .23$).

We tested post hoc the significant multivariate effect of $AUC_{dayCort}$ on future overall CHD risk. Cortisol awakening levels in terms of AUC_{CAR} significantly related to future overall CHD risk (MANCOVA multivariate effects: model 1: $F(3, 99) = 3.17$, $p = .028$, $\eta^2_p = .09$, $f = 0.31$, Wilk´s $\Lambda = .91$; model-2-to-4: $ps \leq .050$) with AUC_{CAR} being associated with the coagulation index (MANCOVA between-subject effects: model 1: $F(1, 101) = 8.28$, $p = .005$, $\eta^2_p = .08$, $f = 0.29$; models 2-to-4: $ps \leq .006$) but not with the inflammatory index ($ps \geq .77$) or prospective changes in tChol/HDL-ratio ($ps \geq .66$). With respect to coagulation measures, AUC_{CAR} predicted greater increases from baseline to follow-up in fibrinogen (regression analyses: model 1: ß = −.17, $p = .082$, $\Delta R^2 = .10$; model 2-to-4: ßs ≥ −.17, $ps \leq .098$, $\Delta R^2 \geq .10$) and in D-dimer (regression analyses: model 1: ß = −.17, $p = .076$, $\Delta R^2 = .06$; model 2-to-4: ßs

$\geq -.17, ps \leq .094, \Delta R^2 \geq .07$) towards a trend for significance. Neither cortisol levels at awaking, slopes, nor evening cortisol levels were associated with future CHD risk ($ps \geq .18$).

Table 4. 2: CHD Risk Factor Changes Between Baseline and Follow-Up

	Total *N* = 106	CHD *n* = 43	HT *n* = 31	NT *n* = 32
Time baseline to follow-up, months	35.54 ± 0.77 (21–63)	33.70 ± 1.11 (21–58)	38.23 ± 1.03 (28–61)	35.41 ± 1.56 (21–63)
Coagulation				
Fibrinogen, g/L	0.10 ± 0.05 (−1.10–2.22)	0.10 ± 0.07 (−0.77–1.27)	0.11 ± 0.07 (−0.78–0.97)	0.10 ± 0.11 (−1.10–2.22)
D-dimer, µg/L	327.10 ± 51.48 (−3678–1575)	228.21 ± 100.78 (−3678–1102)	488.23 ± 61.87 (34–1575)	303.91 ± 80.04 (−1095–1288)
Inflammation				
IL-6, pg/ml	0.07 ± 0.04 (−1.14–1.41)	0.09 ± 0.05 (−0.93–1.41)	0.07 ± 0.05 (−0.48–1.00)	0.06 ± 0.08 (−1.14–1.27)
TNF-α, pg/ml	0.11 ± 0.07 (−3.39–3.23) *n* = 104	0.15 ± 0.09 (−1.04–3.23)	0.30 ± 0.09 (−0.36–2.31)	-0.15 ± 0.15 (−3.39–0.65) *n* = 30
CRP, µg/ml	0.41 ± 0.20 (−8.70–5.63) *n* = 93	0.66 ± 0.25 (−2.11–5.63) *n* = 40	0.15 ± 0.38 (−5.61–3.69) *n* = 28	0.29 ± 0.46 (−8.70–4.04) *n* = 25
tChol/HDL	0.18 ± 0.08 (−1.65–4.38)	0.15 ± 0.11 (−1.65–2.74)	0.09 ± 0.12 (−1.61–1.49)	0.30 ± 0.17 (−1.33–4.38)

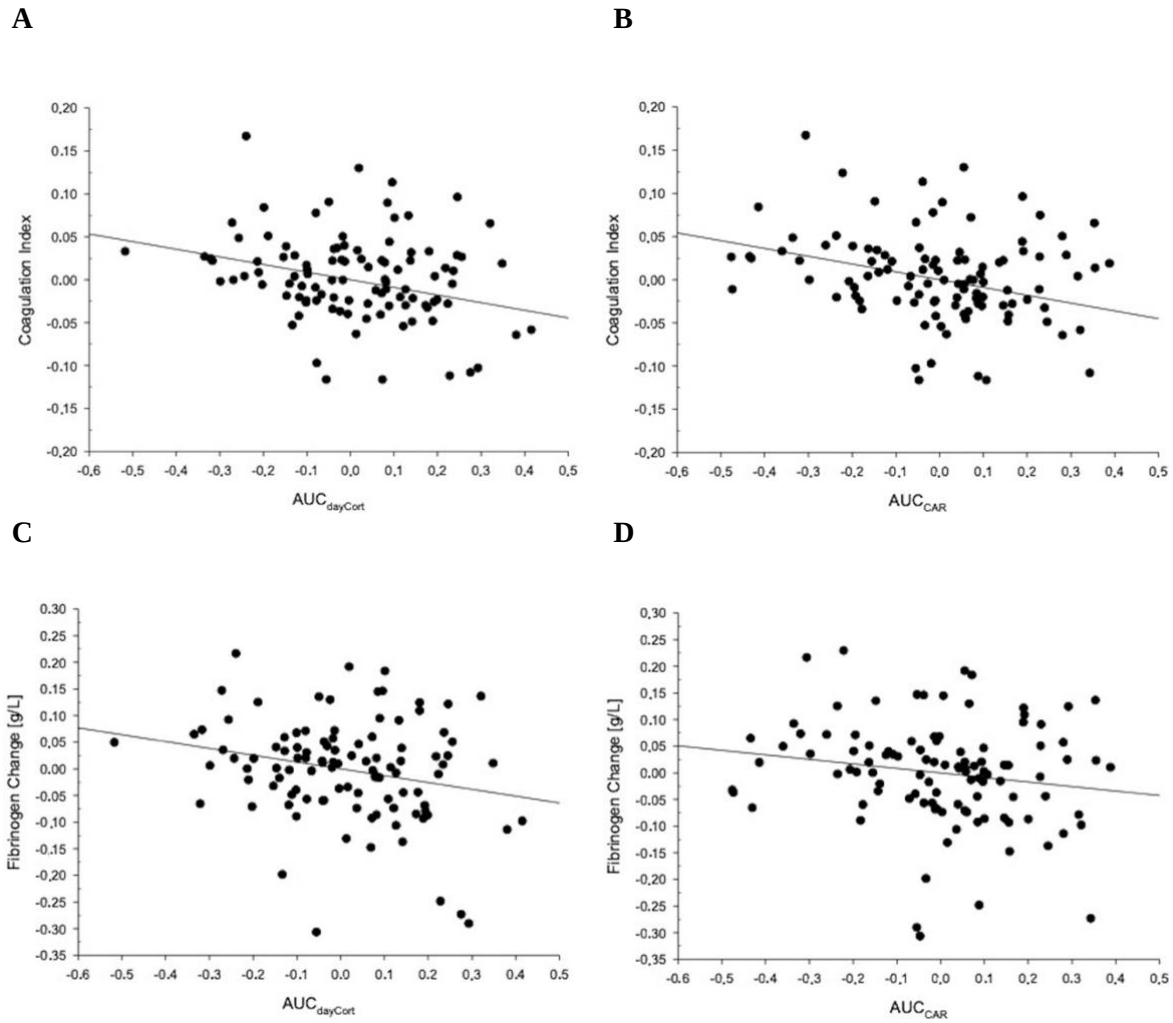
Table 4. 3: Prediction of the Prospective Changes in CHD Risk Factors

Cortisol	Model	Multivariate Tests		Tests of Between-Subjects Effects		
		F	*p*	Index	*F*	*p*
Main analysis	1	3.39	**.021**	Coagulation	7.56	**.007**
$AUC_{dayCort}$				Inflammation	.44	.51
				tChol/HDL	.03	.85
	2	3.27	**.025**	Coagulation	8.27	**.005**
				Inflammation	0.10	.75
				tChol/HDL	0.009	.93
	3	3.22	**.026**	Coagulation	7.70	**.007**
				Inflammation	0.32	.57
				tChol/HDL	0.01	.92
	4	2.90	**.039**	Coagulation	7.55	**.007**
				Inflammation	0.11	.74
				tChol/HDL	0.04	.85
Post hoc	1	3.17	**.028**	Coagulation	8.28	**.005**
AUC_{CAR}				Inflammation	.09	.77
				tChol/HDL	.09	.77
	2	3.09	**.031**	Coagulation	8.30	**.005**
				Inflammation	0.04	.84
				tChol/HDL	0.09	.77
	3	2.98	**.035**	Coagulation	8.10	**.005**
				Inflammation	0.03	.87
				tChol/HDL	0.08	.78
	4	2.70	**.050**	Coagulation	7.79	**.006**
				Inflammation	0.01	.94
				tChol/HDL	0.20	.66
Waking cortisol	1	0.81	.49	Coagulation	2.17	.14
				Inflammation	0.80	.37
				tChol/HDL	0.14	.71
	2	0.83	.48	Coagulation	2.18	.14
				Inflammation	0.91	.34
				tChol/HDL	0.11	.74
	3	1.13	.34	Coagulation	2.75	.10
				Inflammation	1.46	.23
				tChol/HDL	0.01	.91
	4	1.04	.38	Coagulation	2.38	.13
				Inflammation	1.56	.22
				tChol/HDL	0.06	.80
$Slope_{Peak}$	1	1.64	.18	Coagulation	4.70	**.032**
				Inflammation	0.01	.92
				tChol/HDL	0.004	.95
	2	1.62	.19	Coagulation	4.69	**.033**
				Inflammation	0.02	.90
				tChol/HDL	0.02	.90
	3	1.50	.22	Coagulation	4.39	**.039**
				Inflammation	0.02	.88

Cortisol	Model	Multivariate Tests		Tests of Between-Subjects Effects		
				tChol/HDL	0.04	.85
	4	1.25	.30	Coagulation	3.77	.06
				Inflammation	0.08	.78
				tChol/HDL	0.18	.67
$Slope_{Awake}$	1	0.77	.51	Coagulation	1.67	.20
				Inflammation	0.99	.32
				tChol/HDL	.08	.78
	2	0.78	.51	Coagulation	1.64	.20
				Inflammation	1.01	.32
				tChol/HDL	0.10	.75
	3	1.24	.30	Coagulation	2.09	.15
				Inflammation	1.74	.19
				tChol/HDL	0.32	.57
	4	1.18	.32	Coagulation	1.82	.18
				Inflammation	1.97	.16
				tChol/HDL	0.19	.67
Evening cortisol	1	0.76	.52	Coagulation	2.17	.14
				Inflammation	0.002	.96
				tChol/HDL	0.13	.72
	2	0.76	.52	Coagulation	2.11	.15
				Inflammation	0.0001	.99
				tChol/HDL	0.15	.70
	3	0.80	.50	Coagulation	2.00	.16
				Inflammation	0.04	.85
				tChol/HDL	0.28	.60
	4	0.84	.48	Coagulation	1.90	.17
				Inflammation	0.15	.70
				tChol/HDL	0.18	.67

Note. Covariates in model 1: age at baseline, time between baseline and follow-up assessment and medication at follow-up; model 2: additional control for sleep duration and wake-up time; model 3: additional control for study group; model 4: additional control for BMI at baseline as well as BMI and MAP changes between baseline and follow-up. Statistically significant results are highlighted in bold.

Figure 4. 2: Prediction of Future Atherothrombotic Risk by Diurnal HPA-Axis Activity



Note. The figure depicts residuals of the respective dependent and independent variables adjusted for the full set of covariates. MANCOVA post hoc testing, in terms of linear regression analyses, revealed that the coagulation index was significantly associated with (**A**) $AUC_{dayCort}$ ($F = 7.55$; $p = .007$) and (**B**) AUC_{CAR} ($F = 7.79$; $p = .006$). Regression analyses revealed that fibrinogen change from baseline to follow-up was predicted by (**C**) $AUC_{dayCort}$ ($ß = -.26$; $p = .012$) and (**D**) AUC_{CAR} ($ß = -.17$; $p = .098$).

Discussion

The first objective of our study was to cross-sectionally compare diurnal HPA-axis activity between male CHD-patients, HT, and NT at baseline. The novelty of this study is the comparison between HT and CHD-patients. HT and CHD-patients showed lower *overall*

diurnal cortisol saliva concentrations as compared to healthy controls with lowest concentrations in CHD-patients. We found a reduced *CAR* in HT and in CHD-patients as compared to NT corroborating previous findings (Kuehl et al., 2015; Molloy et al., 2008; Pulopulos et al., 2016; Vreeburg et al., 2009; Wirtz et al., 2007). Moreover, HT and CHD-patients did not differ in their CAR. However, regarding *cortisol at awakening*, HT showed highest and NT lowest levels of the three study groups, with CHD-patients showing borderline significantly lower awakening levels compared to HT. These results are in line with previous research, with higher early morning salivary cortisol levels in unmedicated HT compared with healthy controls (Kidambi et al., 2007), whereas medicated HT showed lower early morning cortisol levels compared to NT (Pulopulos et al., 2016). The latter points to a potentially normalizing effect of BP medication on cortisol levels at awakening. In line with this assumption, medicated CHD-patients did not significantly differ from normotensive individuals in their cortisol levels at awakening. Salivary waking or early morning cortisol was not associated with CHD-(measures) in most previous studies including heart patients (Bhattacharyya et al., 2008; Hajat et al., 2013; Nijm et al., 2007). The increased morning cortisol levels in combination with a reduced cortisol response to awakening in our HT may indicate a generally altered HPA-axis activity in the early morning as observed in subjects suffering from a wide range of health problems (Adam et al., 2017; Kudielka & Kirschbaum, 2003). Although, HT and CHD-patients did not differ from NT in their *evening cortisol* levels, patients had significantly higher levels as compared to HT. Evidence from other studies, however, points to cross-sectional associations between higher bedtime (Nijm et al., 2007) or late night (Kidambi et al., 2007) cortisol levels in HT and CHD-symptoms. A potential reason for this discrepancy may be that we assessed cortisol at 22:00h, but not at bedtime or late night levels. Nevertheless, the higher evening levels in our patients may add to adverse cardiac outcomes as observed in other studies (e.g., Hammer et al., 2016). Diurnal cortisol *decline* from

waking to evening ($Slope_{Wake}$) was steepest in HT and flattest in NT, but not independent of covariates, driven by the comparably high awakening levels in HT. Diurnal decline from morning peak to evening ($Slope_{Peak}$) did not differ between groups. Some previous studies pointed to an association between flatter cortisol slopes and greater cardiovascular risk (Matthews et al., 2006; Molloy et al., 2008), so our results, with steeper, and thus more normative (Dmitrieva et al., 2013) cortisol declines in HT as compared to healthy controls, seem unexpected. However, in line with our findings, other studies could not detect any association between diurnal cortisol slope and CHD-measures (Dekker et al., 2008; Hajat et al., 2013). We offer different explanations for these divergencies: First, the observed result of more normative slopes in hypertensive individuals was not independent of covariates. One of the covariates, later awakening time, was borderline significantly associated with steeper slopes in HT as compared to NT ($p = .054$). Second the formulas for the calculation of diurnal slopes differ between studies, rendering comparison of effects difficult (Adam et al., 2017). Since we calculated the slope using evening cortisol levels instead of levels at bedtime, the dynamics of diurnal HPA-axis activity may have been captured incompletely. Third, the observed group differences in diurnal slopes from waking to evening may to some extent be explained by the elevated morning cortisol levels of HT as compared to CHD-patients and normotensive controls. Taken together, we observed group differences in basal HPA-axis activity, with lower CAR and lower overall diurnal cortisol levels in CHD-patients and HT as compared to healthy controls.

We found evidence for aggregated daytime cortisol and CAR levels in predicting overall CHD risk (i.e., dependent variables in the MANCOVA). In detail, we found lower aggregated cortisol levels to predict higher increases in coagulation markers at follow-up, while inflammation markers and blood lipid profile were not associated with basal HPA-axis activity. Moreover, the prospective association between diurnal cortisol secretion and coagulation was

mainly driven by the prediction of fibrinogen increases. So far, only few studies investigated associations between basal HPA-axis and prothrombotic activity: Evidence from cross-sectional studies points to an association between higher cortisol levels (Lippi et al., 2008; von Känel et al., 2008) or dysregulated diurnal cortisol profiles on the one hand (Piazza et al., 2018) and measures including prothrombotic activity on the other hand, which may explain why circulating cortisol had been associated with atherosclerotic vessel damage (von Känel et al., 2008). We found a longitudinal association between lower diurnal HPA-axis activity and higher overall CHD risk increase comprising increases in all three aggregated biological risk factor indices, and in particular with fibrinogen increases. Despite evidence for cross-sectional associations between basal HPA-axis activity and inflammation (Piazza et al., 2018) or hyperlipidemia (Dai et al., 2021; Rosmond & Björntorp, 2000), we could not detect prospective associations between daytime cortisol or CAR levels and changes in inflammation markers and blood lipid profiles. These results are in line with a study in 9-to-10 year-old children where baseline cortisol did not predict blood lipid levels 1 year later (Dai et al., 2021).

The variability between the different measures of cortisol and the outcomes could possibly be attributed to the fact that single cortisol measures may only explain a small proportion of variance and are strongly influenced by situational effects (see, Kirschbaum et al., 1990). As a consequence, it requires repeated diurnal cortisol assessments to be able to detect associations with outcomes.

Our results suggest that dysregulation in terms of reduced CAR and lower overall daytime levels may represent an early indicator for increased cardiovascular risk. However, the clinical utility of the different measures of cortisol and the question about whether the monitoring of the HPA-axis activity facilitates the identification of high-risk individuals needs to be clarified in future studies. Further, the mechanisms underlying the observed HPA-axis activity dysregulation in hypertension and CHD are unclear. Since none of our participants had

a diagnosis of adrenal insufficiency, we consider it unlikely that the observed lower HPA-axis activity in HT and patients result from adrenal insufficiency. One possible explanation may relate to (former) chronic stress experiences during disease development. Chronic stress has been proposed to play a role in both HT and CHD development that has been associated with altered diurnal HPA-axis activity (McEwen, 1998b; Yao et al., 2019). According to the allostatic load model chronic stress causes repeated activation of stress reactions including HPA-axis and sympathetic-adrenal-medullary axis reactivity which accumulate over time leading to compensatory stress system dysregulations in terms of allostatic load (Juster et al., 2010). Given the higher blood pressure and overall SNS activity in hypertension, that notably has been proposed to represent a potential consequence from allostatic load (McEwen, 1998b), the observed lower HPA-axis activity in HT may represent a compensatory allostatic load system dysregulation. Allostatic dysregulation can lead to allostatic overload with tissue and organ damage, including the cardiovascular, the immune, and the metabolic system (Juster et al., 2010). Future studies are needed to further elucidate the role of lifestyle factors (related to allostatic load) in diurnal HPA-axis activity of individuals with elevated CHD risk (see Collomp et al., 2016) and whether our findings in salivary cortisol also apply to cumulative measures of cortisol output such as hair cortisol.

In our prospective analyses, we found that lower aggregated cortisol daytime levels and CAR predicted independent biological CHD risk factors and in particular prothrombotic activity about 3 years later. Studies investigating the effects of glucocorticoid excess (e.g., due to Cushing's syndrome; Isidori et al., 2015) suggest effects on blood coagulation. However, no clear relationship between hypocortisolism and hypercoagulability has yet been established. It remains to be elucidated whether the observed prospective coagulation increases similarly represent a compensatory allostatic load system dysregulation resulting from the HPA-axis dysregulation (Juster et al., 2010; McEwen, 1998b). Also, whether the observed lower CAR,

either alone or combined with the higher prospective coagulation increases, relates to the higher occurrence of myocardial infarctions in the early morning hours (Muller et al., 1989) remains to be elucidated.

Limitations of our study include the relatively high drop-out rate and the wide follow-up range that we mainly attribute to logistic reasons. Participants who dropped out did not substantially differ in their characteristics from those completing the follow-up assessment, except for lower TNF-α and higher CRP levels at baseline (for both, see Supplemental Material). Also, apart from the CAR, we measured cortisol only twice and we assessed evening cortisol and not bedtime levels. Moreover, we cannot completely rule out potential effects of repeated thawing during transportation although salivary cortisol measurements have been shown to be quite robust against repeated freeze-and-thaw-cycles (Garde & Hansen, 2005; Stalder et al., 2016). Moreover, the generalizability of our results is limited to middle-aged men of relatively high socioeconomic status and future studies are needed to further elucidate whether our findings also apply to women (Hajat et al., 2013; Hurwitz Eller et al., 2001) and participants with differing socioeconomic status (Cohen et al., 2006). Also, recruitment via blood donor facilities may interfere with generalizability and we cannot rule out that the use of 24-hr automatic BP measurement would have been even more accurate to diagnose hypertension status compared to the applied two-step assessment procedure including repeated home and study BP measurement. Another limitation of our study relates to the medication of the CHD-patients. First, medication in general can affect salivary cortisol assessment at different levels (e.g., with effects on the composition of the saliva or direct effects on the cortisol synthesis; Granger et al., 2009). Further, the effects of CHD-medication on the different parameters of diurnal cortisol secretion (e.g., CAR) have not been investigated systematically to the best of our knowledge (Wolf et al., 2005), so the comparison between our medicated and unmedicated groups are to be interpreted with caution, as potential medication

effects cannot be ruled out. Also, it is possible, that CHD-medication prevented substantial increases in CHD risk over time in our drug-treated participants. Finally, despite the prospective nature of our study we cannot draw definite conclusions regarding causality as we cannot exclude potential influences by other factors.

Strengths of our study comprise the use of MEMS caps combined with self-recording of sampling times allowing us to ensure the adherence to the study protocol. Further, we controlled for many potentially confounding variables including waking time and sleep duration and cortisol was assessed on 2 consecutive days (Stalder et al., 2016).

In conclusion, we found evidence for a down-regulation of HPA-axis activity in both, CHD and HT. Our results moreover suggest that lower diurnal HPA-axis activity seems to predict poorer cardiovascular health in HT and CHD by promoting a hypercoagulable state. A down-regulation of basal HPA-axis activity may therefore play a role in the pathogenesis and/or progression of atherosclerosis.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics Statement: The studies involving human participants were reviewed and approved by Ethics committee of the Canton of Bern, Switzerland. The patients/participants provided their written informed consent to participate in this study.

Author Contributions: Conceptualization, PHW. Formal analysis, CD. Funding acquisition, PHW and RvK. Investigation, LT, CZ-H and RvK. Methodology, RvK, RW, HS, NM-B and PHW. Project administration, RvK and PHW. Supervision, RvK and PHW. Visualization, CD and PHW. Writing—original draft, CD and PHW. Writing—review and editing, RvK, RW, HS, NM-B and CZ-H. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary Material: The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1080938/full#supplementary-material

Supplementary Material

Supplemental Methods

Study participants: Exclusion criteria. Of a total of 219 participants who provided cortisol with sampling diary data, 12 had to be excluded because of white coat and masked hypertension and another 11 participants had to be excluded as they failed to comply with the cortisol sampling protocol. Furthermore, five participants had to be excluded from the cross-sectional and another two from the longitudinal analyses as they reported symptoms of acute infections on the study day.

Additional information on missing data. In two CHD-patients, information on MI, CABG, and LVEF ≤ 40% was missing. Due to technical problems sodium and potassium could not be analyzed in seven and calcium could not be analyzed in eight HT of the final study sample with two of them having a diagnosis of essential hypertension and being under antihypertensive medication. We measured HbA1c and tChol/HDL-ratio in all participants. Due to technical problems tChol/HDL-ratio was missing in six participants and HbA1c was missing in seven participants. Baseline measurements for IL-6 and TNF-α were missing in three participants. CRP could not be determined in 23 participants at baseline and in 13 participants at follow-up (see Table 4.1-to-2). Reasons for missing data were problems with blood sampling or processing, and insufficient amount of sample for the respective analysis.

Cortisol sampling protocol. We considered cortisol data in our analyses only, if the participants adhered to the study protocol of the respective sampling day with a delay of less than 10 min between awakening and S1, and of less than 75 min between S1 and S5 (Kudielka & Kirschbaum, 2003; Stalder et al., 2016). Eleven participants did not comply with the study protocol on both sampling days and had to be excluded. One participant, who completed only the 1st sampling day did not provide S6 and S7 samples and we replaced these missings by the S5 value.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology, 28*(1), 35-47. https://doi.org/10.1016/S0306-4530(02)00008-2

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D. H., Miller, R., Wetherell, M. A., Lupien, S. J., & Clow, A. (2016). Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology, 63*, 414-432. https://doi.org/10.1016/j.psyneuen.2015.10.010

Assays for the prospective CHD risk assessment and HbA1c.

***Prothrombotic Measures*.** Venous blood was drawn into polypropylene tubes containing 3.8% sodium citrate (Sarstedt, Nümbrecht, Germany). Citrate tubes were immediately centrifuged for 20 min at 4 °C at 2000g and plasma was pipetted into aliquots. D-dimer was analyzed using a particle-enhanced immunoturbidimetric assay for the quantitative determination of D-dimers in human plasma (INNOVANCE® D-Dimer, Siemens Healthcare GmbH, Erlangen, Germany) on a Sysmex CS-5100 (Sysmex Europe, Norderstedt, Germany). Plasma fibrinogen levels were determined by a routine clotting assay applying standard quality procedures following the Clauss method. The intra- and inter-assay coefficients of variation were ≤ 7.9%.

Blood Lipids. We measured tChol and HDL from heparine-coated monovettes (Sarstedt monovette orange). Analyses were performed using in vitro assays (enzymatic colorimetric) for the quantitative determination of blood lipids in human plasma (Roche, Mannheim, Germany) on a Roche/Hitachi Cobas C Analyzer (Roche, Mannheim, Germany). Mean inter- and intra-assay CVs were ≤ 1.2% and ≤ 2.5% respectively.

Pro-inflammatory Measures. For the determination of IL-6, TNF-α, and CRP venous blood was drawn in EDTA-coated monovettes (Sarstedt, Nümbrecht, Germany), and immediately centrifuged for 10 min at 2000g and 4 °C. Obtained plasma was stored at −80 °C until analysis. IL-6 and TNF-α levels were determined with a high sensitivity chemiluminescence sandwich immunoassay (Meso Scale Discovery [MSD], Rockville, USA). CRP was determined using a high-sensitive enzyme immunoassay (ELISA, IBL Hamburg, Germany). For IL-6, mean inter- and intra-assay CVs were ≤ 7.3% and ≤ 4.5%. For TNF-α inter- and intra-assay CVs were ≤ 10.1% and ≤ 3.4% respectively. For CRP mean intra- and inter-assay CVs were < 5.1% and 6.1% respectively.

HbA1c. To measure HbA1c, venous blood was drawn into EDTA-coated monovettes, and analyses were performed with in vitro assays for the quantitative determination of HbA1c IFCC (mmol/mol) in whole blood (Tina-quant®, Roche, Mannheim, Germany) using Roche/Hitachi Cobas C Systems (Roche, Mannheim, Germany). Mean inter- and intra-assay CVs were ≤ 1.6% and ≤ 2.0% respectively.

Log-transformed data. All measures showing a skewed distribution (cross-sectional analyses: age, BMI, fibrinogen, D-dimer, tChol/HDL-ratio, IL-6, TNF-α, CRP, cortisol concentrations, $Slope_{Awake}$, mean awakening time, sleep duration, systolic blood pressure measured at home for screening and HbA1c; prospective analyses: AUC_{TC}[3], AUC_{CAR}, evening cortisol concentrations, mean awakening time, time between baseline and follow-up assessment, BMI change, tChol/HDL-ratio change, as well as inflammatory and coagulation indexes, D-dimer and fibrinogen changes) were log-transformed.

[3] $AUC_{TC} = AUC_{dayCort}$.

Supplemental Tables

Supplemental Table S4.1

Medication

[%]	CHD *n* = 83	HT *n* = 12
ACE inhibitors	32 (38.6)	4 (33.3)
AT2 antagonists	30 (36.1)	7 (58.3)
Anticoagulant	81 (97.6)	-
Alpha-blockers	3 (3.6)	1 (8.3)
Alpha-&beta-bl.	4 (4.8)	-
Beta-blockers	50 (60.2)	-
Antiarrhythmics	3 (3.6)	-
Lipid-lowering drugs	78 (94.0)	1 (8.3)
Antidiabetic medication	9 (10.8)	-
Insulin	1 (1.2)	-
Parkinson´s disease medication	1 (1.2)	-
Antidepressants	4 (4.8)	-

Supplemental Table S4.2

Comparison of Baseline Group Characteristics Between Participants Completing Follow-Up Assessment and Drop-Out Participants

	Follow-Up (*n* = 106)	Drop-Out (*n* = 85)	*p*
Age, years	57.49 ± 1.13 (28–80)	57.58 ± 1.47 (21–85)	.81
BMI, kg/m^2	27.03 ± 0.34 (19.78–38.90)	27.65 ± 0.44 (21.93–46.44)	.28
Study MAP, mmHg	102.72 ± 1.12 (75.33–139.89)	102.89 ± 1.21 (79.83–132.00)	.92
Study SBP, mmHg	140.09 ± 1.63 (109.33–189.67)	140.81 ± 1.65 (112.50–187.33)	.76
Study DBP, mmHg	84.04 ± 1.11 (58.33–115.00)	83.93 ± 1.18 (62.67–108.00)	.95
Fibrinogen, g/L	2.69 ± 0.05 (1.65–4.46)	2.75 ± 0.06 (1.47–4.25)	.50
D-dimer, µg/L	513.68 ± 52.03 (45–4765)	594.75 ± 74.90 (167–5177)	.11
IL-6, pg/ml	0.56 ± 0.04 (0.03–3.61)	0.54 ± 0.03 (0.09–1.57) *n* = 82	.93
TNF-α, pg/ml	2.10 ± 0.07 (0.80–4.91)	1.90 ± 0.07 (0.71–5.11) *n* = 82	.020
CRP, µg/ml	2.02 ± 0.19 (0.07–9.59) *n* = 97	2.76 ± 0.22 (0.35–11.55) *n* = 71	< .001
tChol/HDL	3.47 ± 0.09 (1.73–6.31)	3.56 ± 0.11 (1.76–6.41) *n* = 79	.60
Cortisol at awakening, nmol/L	4.42 ± 0.27 (0.02–14.83)	4.71 ± 0.32 (0.38–18.00)	.32
Cortisol at 16:00h, nmol/L	1.79 ± 0.11 (0.14–6.51)	1.85 ± 0.12 (0.06–7.38)	.73
Cortisol at 22:00h, nmol/L	0.77 ± 0.07 (0.04–6.10)	0.87 ± 0.08 (0.07–3.22)	.16
Cortisol Slope$_{Peak}$	−0.48 ± 0.03 (−1.63–0.18)	−0.48 ± 0.03 (−1.23–0.18)	.97
Cortisol Slope$_{Awake}$	−0.23 ± 0.02 (−0.87–0.17)	−0.25 ± 0.02 (−1.00–0.18)	.49

General Discussion

The present thesis aimed to investigate the short-term and long-term effects of acute stress and the HPA-axis (re)activity and to elucidate the underlying biological mechanisms. More specifically, the first two studies of the thesis focused on the short-term effects of acute stress and the role of stress-induced cortisol reactivity on concentration performance and eating behavior in healthy participants. The third and fourth studies aimed to shed light on the clinical relevance of the blood lipid reactivity to acute stress and the basal HPA-axis activity in healthy normotensive and hypertensive individuals (Studies 3 and 4) as well as CHD-patients (Study 4). In the following section, I briefly summarize the methods and major findings of the studies of the thesis project and discuss our research approach. To conclude, possible implications of our findings will be considered.

Summary and Discussion of the Major Study Findings and Designs

Short-Term Effects of Acute Stress and HPA-Axis Reactivity

The first study investigated the effects of acute psychosocial stress on *concentration performance* in healthy young men. The study placed particular emphasis on the underlying endocrine mechanisms in terms of acute stress-induced HPA-axis reactivity assessed by means of cortisol increases (i.e., changes between baseline and peak levels). Most previous studies compared performance in a concentration task between an experimental group (exposed to acute stress) and a non-stress group (Banks et al., 2014; Scholz et al., 2009; von Dawans et al., 2012). Another study investigated individual changes prior to and following stress induction, notably without a control condition (Olver et al., 2015). We, therefore, compared performance changes in the d2 Test of Attention between a stress group (exposed to the TSST) and a control group (simultaneously doing a non-stressful task). Concentration performance was assessed prior to (at baseline) and 30 min after the respective experimental manipulation. Compared to

the participants of the control group, stressed participants improved significantly more in their concentration performance between the initial assessment and the post-task assessment. These results are consistent with a previous study reporting beneficial effects of a mild non-mental stressor (i.e., CPT) on selective attention performance (Shields et al., 2019). Thus, a certain level of acute stress, independent of its nature, could be favorable for concentrated performance when carrying out specific tasks. This result is in line with the capacity theory according to which individuals have more attentional capacity at their disposal when completely alerted (Kahneman, 1973; Moran, 2012). Similarly, it has been suggested, that an optimal level of arousal is vital for attention (Esterman & Rothlein, 2019) supporting the assumption of an inverted-U shaped association between acute stress and cognitive functioning (Sapolsky, 2015). It is worth noting that most studies investigating acute psychosocial stress effects on (different measures of) concentration performance found either adverse (Olver et al., 2015; Scholz et al., 2009) or no effects (Banks et al., 2014; von Dawans et al., 2012), emphasizing the need for further research. As previously described, these studies vary considerably in their methodological approaches (e.g., regarding the used tests to quantify attention or the timing after stress induction) and future research projects should build on comparable methods to fully uncover and understand short-term acute stress effects on concentration performance.

To capture potential underlying mechanisms, salivary cortisol (in addition to salivary alpha-amylase, state anxiety, and anticipatory cognitive stress appraisal; see below) was repeatedly assessed at baseline and up to 60 min after each condition. We found that concentration performance enhancement was associated with lower (stress-induced) cortisol changes, as it has been suggested in a preceding study using cortisol administration (Hsu et al., 2003). This result could be interpreted as further evidence for the inverted-U shaped association between stress and cognitive performance, with the transition from mild to moderate stress—that may be accompanied by stronger cortisol increases (e.g., Skoluda et al.,

2015)—being associated with performance decreases (Sapolsky, 2015). It is, however, important to note that due to the study design, conclusions regarding the potential inverted-U shaped associations between acute stress and concentration performance can only be drawn with caution. Another possible explanation for the observation is that individuals with strong cortisol responses to the TSST may have a higher sensitivity to social evaluation and thus an increased vigilance to threat (Lopez-Duran et al., 2020) which might reduce the concentration performance in a cognitive task. In contrast, two previous studies did not detect a link between stress-induced cortisol reactivity and attention (Olver et al., 2015; Shields et al., 2019). We assumed that the methodological differences between these two studies and our investigation (e.g., inclusion of both sexes without sufficient statistical control, differences in cortisol sampling protocol with low or missing cortisol increases after stress induction; Olver et al., 2015; Shields et al., 2019) may be responsible for the divergent results.

Although the present thesis focused explicitly on the biological mechanisms underlying the effects of stress, it is important to note that the first study indicated a positive association between concentration performance and state anxiety. This finding points to the beneficial effects of acute stress and stress-induced anxiety on concentration performance. In fact, anxiety can lead to arousal and increased vigilance (Grillon, 2002). Although anxiety has also been suggested to increase attention toward threats, individuals with higher state anxiety might rely on compensatory strategies, e.g., making greater effort when facing anxiety-inducing situations (Eysenck et al., 2007). No associations between concentration performance and SAM-axis stress reactivity or anticipatory cognitive stress appraisal were found in the study, which we explained by the timing of the second concentration performance assessment. Indeed, the second assessment was timed at 30 min after the completion of the TSST or the control task respectively, when SAM-axis stress reactivity has recovered reaching baseline levels and anticipatory cognitive stress appraisal may have already subsided. Together, the differing

effects of the HPA-axis stress reactivity (in terms of cortisol) and anxiety suggest "a potential modulating role of specific psycho-emotional and physiological factors with opposite effects" (Degroote et al., 2020, p. 96).

The second study focused on *eating behavior* after acute psychosocial stress. More specifically, we were interested in the underlying endocrine mechanisms in terms of stress-induced cortisol reactivity in healthy men and women. The investigation of potential sex differences in the mechanisms of eating after stress was a further research aim, as women generally eat healthier than men (de Castro & Kreitzman, 1985; Rolls et al., 1991; Wardle et al., 2004; Westenhoefer, 2005), but show at the same time a higher total prevalence of obesity (Chooi et al., 2019). All participants completed the TSST before they rated ice cream in the BTT starting 15 min after stress cessation. Cortisol and the satiety hormone CCK were repeatedly assessed from saliva samples as well as perceived hunger prior to and up to 60 min after acute psychosocial stress induction.

A higher immediate total cortisol stress reactivity (aggregated as AUC_G from baseline to stress-induced peak levels) predicted lower hunger corroborating previous studies that pointed to reduced hunger (e.g., van Strien et al., 2014) after acute stress. We interpreted lower hunger after acute stress as a part of the fight-or-flight response (e.g., Kiessl & Laessle, 2016; Torres & Nowson, 2007), including the down-regulation of processes that are not essential for survival (e.g., Chrousos & Gold, 1992). The results suggested that this down-regulation may be regulated by stress-induced cortisol levels. However, there was no significant relationship between the immediate total cortisol stress reactivity and the amount of ice cream consumed during the taste test following stress induction. We discussed that the time-limited BTT may have influenced the subjects' eating behavior, as they may not have felt that they could eat the amount of food they actually wanted. In contrast to previous evidence suggesting that cortisol may be associated with the regulation of appetite and satiety hormone secretion, both on a basal

level (Kuckuck et al., 2023) and in response to stress (Rouach et al., 2007), immediate total cortisol stress reactivity was not related to CCK release in our study. Based on our study results and previous findings pointing to an absence of associations between satiety hormones and stress-induced cortisol increases (Tomiyama et al., 2012), we assumed that the inhibiting effects of the immediate total cortisol stress reactivity on hunger after acute stress are regulated at a central level, as described in more detail below (Alhabeeb et al., 2021; Huda et al., 2006; Sam et al., 2012). It should further be mentioned that the design did not include a corresponding control condition without stress induction, which would have allowed us to investigate direct stress effects on the amount of food intake, hunger, and CCK secretion.

In terms of sex differences, women consumed less ice cream after experiencing acute stress and consistently reported a lower sensation of hunger compared to men, as it has been suggested by previous studies (de Castro & Kreitzman, 1985; Oliver et al., 2000; Rolls et al., 1991). Also in this regard, it would have been beneficial to include a non-stress control condition in our study design to determine if women also consume less food than men without stress induction. Moreover, despite our recruiting protocol including only women without oral contraceptive use during the luteal phase of their monthly cycle, women had lower overall cortisol levels as well as a lower immediate total cortisol stress reactivity than men. No differences between men and women were, however, found in the kinetics of cortisol stress reactivity. The kinetics of CCK during the experimental procedure differed between men and women. Particularly following the TSST, but also during the remaining experimental procedure, CCK continuously declined in women. In contrast, men´s CCK levels were reactive to acute psychosocial stress and tended to increase after the taste test.

Indicating negative associations between cortisol and hunger on the one hand as well as sex differences in cortisol and CCK on the other hand, the results of the second study suggest

a possible explanation for the reported higher risk for stress-induced eating in women (e.g., Cotter & Kelly, 2018).

Taken together, the first two studies of the thesis provide further evidence for the important *short-term effects* of acute psychosocial stress in our everyday lives, which are partially modulated by stress-induced HPA-axis reactivity. More specifically, the study findings suggest that stress-induced cortisol increases can affect concentration performance and the perception of hunger. These observations could be explained by the effects of cortisol in the brain. Cortisol can easily cross the blood-brain barrier (Sandi, 2013), where it can elicit numerous cognitive effects via binding on GR and MR (Vogel et al., 2016). Under conditions of acute stress, the MR promote a shift in resources from higher-order processes towards more efficient habit systems and support increased vigilance (Vogel et al., 2016). Moreover, it has been suggested that cortisol may contribute to a decreased activity of the cuneus, which is involved in visual processing (Henckens et al., 2012). These observations point to a reduction in bottom-up attentional processing (Henckens et al., 2012) that may affect concentration performance. These findings support our results regarding the negative associations between stress-induced cortisol and concentration performance. With respect to cortisol effects on perceived hunger, stress-induced cortisol increases may interact with orexigenic and anorexigenic neuropeptides of the ARC (Maniam & Morris, 2012). Based on findings from a rodent study, we speculated that the stress-induced HPA-axis reactivity may reduce the expression of orexigenic neuropeptides, possibly explaining the observed negative association of cortisol and hunger (Ans et al., 2018; Chagra et al., 2011). In contrast, cortisol has also been reported to influence central reward pathways that may promote stress eating (Adam & Epel, 2007). These results emphasize the complexity of the effects of cortisol on central processes and the associated cognitive and behavioral outcomes. For this reason, further studies for example using receptor blockades (e.g., Cornelisse et al., 2011) or investigating neural effects

of cortisol in imaging studies (e.g., Hsu et al., 2003) are needed to elucidate these central mechanisms that are under the control of cortisol.

The short-term adaptive responses to stress are assumed to fulfill first and foremost a protective role for the organism (Dhabhar, 2018; McEwen & Seeman, 1999). With regard to the adaptive function of cortisol stress reactivity, the two studies came to seemingly contradictory conclusions. Higher (stress-induced) cortisol changes were associated with a worsening of the concentration performance in the d2 Test of Attention, which could be interpreted as an inadequate adaption to stress. This dampened improvement of individuals with high cortisol (stress) levels might be explained by their assumed greater stress-induced vigilance to threat (Lopez-Duran et al., 2020) which could be a helpful adaption to stress under certain conditions. Nevertheless, it is important to note that in our study greater state anxiety was associated with improved concentration performance. This finding highlights the importance of considering psychological factors and their interactions with endocrine mechanisms when examining the short-term effects of acute stress. Concerning eating behavior after stress, it has been suggested that a reduced feeling of hunger after acute stress reflects an appropriate and adaptive reaction, contributing to the fight-or-flight response (e.g., Kiessl & Laessle, 2016; Torres & Nowson, 2007). Since higher cortisol levels were related to lower perceived hunger in our study, the effects of cortisol with respect to eating behavior after stress may be interpreted as adaptive. It can therefore cautiously be assumed that cortisol stress reactivity leads to adaptive responses in healthy individuals.

Regarding the *methodological approaches* of the first two studies of the thesis, the frequently described utility of the TSST for the investigation of acute stress and its underlying mechanisms has been confirmed (e.g., Allen et al., 2014; Dickerson & Kemeny, 2004). High levels of cortisol were induced in both studies. Since we observed generally lower cortisol levels in women (despite the control of the menstrual cycle phase and the exclusion of oral

contraceptives), the second study emphasized the modulating role of sex regarding the underlying endocrine mechanisms of stress effects. Future research should therefore always include both, men and women, as research findings in a male study sample may not necessarily apply to women.

I have focused on the endocrine mechanisms (i.e., cortisol stress reactivity) behind the short-term effects of acute stress when writing the thesis. However, the first study revealed that psychological factors related to stressful experiences, such as anxiety, could also have modulating properties that also need to be taken into account in following research projects. Moreover, we did not consider confounding factors in terms of individual characteristics that are discussed to influence concentration performance or eating behavior respectively. For example, the ability to concentrate has been associated with motivation (Esterman et al., 2016; Oken et al., 2006) and expertise (Sörqvist et al., 2016; Sörqvist & Marsh, 2015). In addition, numerous studies reported that the association between stress (and stress-induced cortisol levels) and eating could be affected by restrained eating (Kandiah et al., 2008; Newman et al., 2007; Wallis & Hetherington, 2009; Zellner et al., 2006), emotional eating (Oliver et al., 2000; Raspopow et al., 2014; van Strien et al., 2012; van Strien et al., 2014; Wallis & Hetherington, 2009), or stress eating (Epel et al., 2004; Sproesser et al., 2014).

Further, the studies were conducted under highly standardized conditions reducing the ecological validity of our results. The performance in cognitive tests provides only a limited insight into the general cognitive performance in everyday settings (Spooner & Pachana, 2006). Indeed, the need for concentration is largely determined by the difficulty of the current task and task demands depend on the perceptual, sensory, and cognitive load (Sörqvist et al., 2016). Given the high cost of failures related to attention and concentration deficits, for example in the medical context (Murphy et al., 2016), a better understanding of acute stress effects on sustained attention across different task demands may be of particular interest. Regarding stress

effects on eating, future studies should also take into account external influences, including environmental, e.g., food availability, or social factors such as the presence of others, that may impact food intake (Torres & Nowson, 2007).

Taken together, the first two studies of the thesis add new insights to the vast field of research regarding the short-term effects of acute stress and the mediating role of stress-induced cortisol increases. At the same time, numerous questions arose that emphasized the need for further studies.

Long-Term Effects of Acute Stress and HPA-Axis Activity

The third study aimed to compare the hypothesized *blood lipid reactivity* to acute psychosocial stress between healthy normotensive and essential hypertensive men and to examine possible associations between blood lipid stress reactivity and future CHD risk. We first tested whether acute psychosocial stress employing the MIST would lead to changes in different blood lipid markers (i.e., TC, HDL-C, LDL-C, TG, and TC/HDL-C ratio) in all participants. Similar to previous studies (Stoney, Bausserman, et al., 1999; Stoney & Finney, 2000; Wirtz et al., 2009), significant hemoconcentration-corrected responses in HDL-C, LDL-C, and TC/HDL-C ratio scores were detected following the MIST. We assumed that blood lipid reactivity may relate to the stress-induced increases in circulating catecholamines, as it has been suggested in a preceding study of our research group (Arner, 2005; Wirtz et al., 2009). In contrast to other blood lipid markers, TC levels were not reactive to stress and TG levels decreased in response to the MIST. As TG are an important source of energy, we hypothesized that TG may be metabolized after the increased energy expenditure in response to acute stress (Hitze et al., 2010; Stoney, 2017). Similar to the study of our research group (Wirtz et al., 2009), hypertensive participants exhibited a more substantial increase in TC/HDL-C ratio scores and a less pronounced decrease in TG levels after acute psychosocial stress than

normotensive participants. Previous research has shown that hypertensives show hyperreactivity—particularly in response to relatively potent stressors like the TSST or the MIST—in stress-reactive physiological systems, including lipid metabolism (e.g., Walther & Wirtz, 2023). Several mechanisms for this stress hyperreactivity in hypertensives have been proposed (Walther & Wirtz, 2023). Hypertensives and normotensives seem to differ on a cognitive-emotional level, including differences in the perception and evaluation of stressors as well as in the related affective responses that render hypertensives more vulnerable to stress effects (Walther & Wirtz, 2023). Moreover, the physiological stress hyperreactivity of hypertensives has also been related to functional alterations on the hypothalamic and brainstem level, leading for example to an increased NE release (Walther & Wirtz, 2023). These alterations have been linked to the peripheral regulation of several intermediate biological risk factors (Walther & Wirtz, 2023). With respect to the observed differences in blood lipid stress reactivity between normotensives and hypertensives, the latter may have had a higher blood lipid reactivity due to the metabolic effect of catecholamine spillover in response to acute psychosocial stress (Arner, 2005; Wirtz et al., 2009).

Regarding the clinical relevance of blood lipid stress reactivity, we tested whether total aggregated TC/HDL-C stress reactivity (i.e., AUC_G) would predict prospective CHD risk, measured by means of changes in TC/HDL-C ratio, IL-6, D-dimer, and HbA1c between baseline and follow-up. In line with previous evidence linking basal TC/HDL-C levels to future CHD risk (Calling et al., 2021), TC/HDL-C stress reactivity predicted overall future CHD risk in terms of prospective changes in intermediate biological CHD risk factors. Post hoc analyses revealed (borderline) significant associations with prospective increases in HbA1c, D-dimer, and IL-6, and partly with TC/HDL-C ratio changes. We discussed that the transient rise in blood lipids may contribute to atherosclerotic processes, leading to inflammatory and atherothrombotic changes (Libby, 2021; Ross, 1999). This may account for the observed

prospective association between blood lipid stress reactivity and IL-6 and D-dimer increases. Further studies are needed to shed light on the potential mechanisms that could explain the suggested prospective association between blood lipid stress reactivity and HbA1c. Regarding our findings on the possible association between blood lipid stress reactivity and prospective elevations in TC/HDL-C ratio, the accumulation of frequent stress experiences may lead to chronically increased blood lipid levels (Marcondes et al., 2012; McEwen, 1998b; Steptoe & Brydon, 2005).

The fourth study investigated whether alterations in the *basal HPA-axis activity* might contribute to future CHD risk. In more detail, the first objective was to compare basal HPA-axis activity, in terms of the diurnal cortisol rhythm, between male CHD-patients, essential hypertensives, and healthy normotensives. Compared to the healthy controls, CHD-patients and hypertensives exhibited reduced overall cortisol concentrations over the day. These differences were particularly evident in their morning levels. In detail, hypertensives and CHD-patients had a similar CAR, but the two groups showed a lower CAR than normotensive individuals as it has been suggested in previous studies (Kuehl et al., 2015; Molloy et al., 2008; Pulopulos et al., 2016; Vreeburg et al., 2009; Wirtz et al., 2007). Moreover, hypertensive participants presented the highest cortisol levels upon awakening, while individuals within the normotensive BP range showed the lowest levels at this point in time. This HPA-axis morning activity in hypertensives, with elevated cortisol levels after awakening but reduced CAR, is similar to cortisol profiles observed in other health disorders (Adam et al., 2017; Kudielka & Kirschbaum, 2003). Regarding evening cortisol levels, hypertensives and CHD-patients had similar concentrations to normotensives. However, CHD-patients showed (borderline significantly) higher levels of cortisol at this timepoint compared to hypertensives. Hypertensive participants had the steepest waking-to-evening cortisol slope, while normotensives showed the flattest decline. With respect to previous evidence linking flatter

diurnal slopes to increased cardiovascular risk (e.g., Molloy et al., 2008), we explained this unexpected result by methodical issues (e.g., the results were dependent on covariates, and were mainly determined by the high levels at awakening in hypertensive participants). Interestingly, Adam et al. (2017) discussed that heightened morning cortisol levels, together with a steep slope, may lead to increased anxiety. It needs to be elucidated whether this potential association may contribute to the stress hyperreactivity of hypertensives. To conclude, we found significant differences in basal HPA-axis activity between healthy, high-risk and diseased individuals. Together, CHD-patients and hypertensives had a lower CAR and reduced basal HPA-axis activity over the day in comparison to healthy participants with normal BP. We assumed that these observed differences between high-risk individuals (i.e., hypertensives) and CHD-patients on the one hand and healthy normotensives on the other hand may be attributable to chronic stress exposure during the development of the disease (McEwen, 1998b; Yao et al., 2019). In line, the above-described stress hyperreactivity of hypertensives has been linked to perceived chronic stress (Walther & Wirtz, 2023). Moreover, it has been suggested that individuals with advanced atherosclerosis may become more easily exhausted, which may cause them to perceive their environment as more stressful (Steptoe & Kivimäki, 2012). Chronic stress experiences in turn have repeatedly been associated with altered diurnal cortisol profiles, including the hypoactivity of the HPA-axis (e.g., Adam et al., 2017; Guilliams & Edwards, 2010; Kudielka et al., 2012; Zilioli et al., 2018). It has been suggested that the initial basal HPA-axis hyperactivity in response to chronic stress is followed by a down-regulation to protect brain functioning and metabolism (Guilliams & Edwards, 2010).

To investigate the predictive value of the basal HPA-axis activity with respect to CHD risk, we tested for prospective associations with intermediate biological CHD risk factors. The outcome variables encompassed aggregated changes from baseline to follow-up in coagulation (D-dimer and fibrinogen) and inflammatory (IL-6, TNF-α, CRP) markers as well as TC/HDL-

C ratio. Lower total cortisol secretion over the day and lower CAR predicted CHD risk approximately 3 years later. In this way, alterations in the basal HPA-axis activity may represent an important pathway for the effects of stress on cardiovascular health (Knight et al., 2021). Although cross-sectional studies suggested a link between basal HPA-axis activity and inflammatory markers (Knight et al., 2021; Piazza et al., 2018) or dyslipidemia (Dai et al., 2021; Rosmond & Björntorp, 2000), we did not find evidence for corresponding longitudinal associations. However, the analyses revealed a negative association between the basal HPA-axis activity and prospective changes in coagulation, which was mainly explained by the effects on fibrinogen increases. Interestingly, cross-sectional studies have suggested that elevated morning serum cortisol levels are associated with increased prothrombotic activity (Lippi et al., 2008; von Känel et al., 2008). Further, glucocorticoids may improve the production of hemostatic agents on cellular and protein levels (von Känel et al., 2008). Accordingly, heightened cortisol levels, e.g., due to Cushing's syndrome have also been related to alterations in hemostatic processes (Isidori et al., 2015). However, the mechanisms underlying the observed link between low cortisol levels over the day and hypercoagulability in our study remain to be elucidated.

Taken together, the third and the fourth study provide additional evidence for the *long-term effects* of acute stress and the basal HPA-axis activity with respect to CHD risk (e.g., Kivimäki et al., 2012; Rosengren et al., 2004; Steptoe & Kivimäki, 2012). In more detail, our results point to an altered regulation of the stress system in hypertensives and CHD-patients. We observed a higher blood lipid stress response in hypertensives and a lower basal activation of the HPA-axis in hypertensives and CHD-patients, both compared to healthy normotensives. These observed dysregulations in hypertensives and CHD-patients were associated with long-term CHD risk in terms of prospective increases in intermediate biological risk factors. In contrast, healthy normotensives had more normative profiles in their blood lipid stress

reactivity and basal HPA-axis activity and were, therefore, less vulnerable in the long term. Together these findings support the assumption that stress and the associated changes do not necessarily favor the onset of the disease itself but may play a more crucial role in the progression and outcome of cardiovascular diseases (Kivimäki & Steptoe, 2018). In addition, the prospective effects of both elevated blood lipid stress reactivity on the one hand and lower basal HPA-axis activity on the other hand on intermediate biological CHD risk factors provide mechanistic insights on the health-related effects of stress and allostasis. The studies showed that a higher blood lipid stress reactivity was associated with prospective increases in HbA1c, D-dimer, and IL-6, while a lower diurnal HPA-axis activity predicted increases in fibrinogen. These prospective associations could be interpreted as the result of allostatic overload: Under conditions of repeated SAM-axis and HPA-axis activation, interrelated biological systems may overcompensate and ultimately become disrupted, rendering the organism susceptible to stress-related medical conditions such as hypertension and CHD (Juster et al., 2010). In more detail, the dysregulation of the stress system is compensated by associated biological systems that adapt their operating ranges leading to a prodromal stage (Juster et al., 2010). This stage can be characterized by subclinical levels of metabolic, cardiovascular, and immune parameters (Juster et al., 2010; McEwen & Seeman, 1999) that have—at least partially—been observed in our prospective analyses.

With respect to the *methodological approaches* of the third and the fourth study, different limiting factors and implications for future studies became apparent. We found evidence for an altered regulation of stress-reactive systems (in terms of higher blood lipid stress reactivity and lower diurnal HPA-axis activity) in hypertensives and/or CHD-patients. These observations, pointing to dysregulated stress systems in hypertensives and CHD-patients, may result from psychological and biological mechanisms (Walther & Wirtz, 2023) which are associated with allostatic load (McEwen, 1998b; McEwen & Seeman, 1999).

However, our data did not allow us to draw any conclusions regarding the sources of the observed alterations in hypertensives and CHD-patients. As described earlier, a complex interplay of biological and psychosocial factors may explain these dysregulations. Future studies should therefore also take into account other stress-related psychosocial CVD risk factors, including characteristics of an individual's social environment, personality, and negative affect (von Känel, 2012). A better understanding of the psychosocial mechanisms underlying health-related risks may facilitate the deduction of individual intervention strategies. Moreover, as suggested by the second study of the thesis, stress may induce changes in health behaviors (Cohen et al., 2016; O'Connor et al., 2021; Schneiderman et al., 2005) and could thereby indirectly contribute to an increase in CHD risk factors (e.g., Hill et al., 2022; Tomiyama, 2019). Future research should consider this aspect as well.

In the two studies, slightly differing parameters and measures (change values vs. indices formed from the change values of the intermediate biological CHD risk factors) were used to define future CHD risk. This approach renders the direct comparison of the prospective findings of both studies more difficult and should be solved differently in future studies. Nevertheless, investigating CHD risk by means of intermediate biological risk factors can be considered as a strength of the study design. First, intermediate biological CHD risk factors are inexpensive biomarkers (Ridker et al., 2001) with predictive value for CHD (e.g., Rodondi et al., 2010). Second, they permit insights into the processes underlying atherosclerosis (Aday & Ridker, 2019; Ridker et al., 2001; Rodondi et al., 2010). Third, in contrast to disease endpoints, preclinical parameters, such as the intermediate biological risk factors, allow the investigation of the gradual development of pathological processes not only in diseased but also in healthy individuals (Treiber et al., 2003).

The third study demonstrated that psychosocial stress tests used in functional imaging settings, such as the MIST (Dedovic et al., 2005), are suitable for examining the impact of

acute stress on a physiological level. Despite possible ethical concerns, the investigation of neuronal stress processing in CHD-patients could provide important insights into further mechanisms underlying their increased risk when facing acute stress (Bremner et al., 2023).

My conclusions regarding the association of dysregulated stress-reactive systems and CHD risk are limited to the observations in blood lipid stress reactivity on the one hand and diurnal HPA-axis activity on the other hand. Future studies should extend this research approach to the stress reactivity and basal regulation of the same system. In addition, further research is required to test whether these observations can also be extended to other stress-reactive systems, such as the renin-angiotensin-aldosterone system. In line, evidence suggests an aldosterone hyperreactivity to acute psychosocial stress (Gideon et al., 2021) and an altered basal aldosterone secretion in hypertensives (Gideon et al., 2024) as compared to normotensives. Whether these dysregulations of the renin-angiotensin-aldosterone system also apply to CHD-patients and whether they predict future CHD risk remains to be elucidated.

Because of the lack of female representation in cardiovascular research (Kim et al., 2008), future studies should be extended to female populations. Potential sex differences in the underlying mechanisms of long-term effects of acute stress and HPA-axis activity may not be unlikely, since women generally differ from men, both in their stress hormone levels (e.g., Kajantie & Phillips, 2006) and in the circulating biomarkers of CVD (Lau et al., 2019).

Implications and Future Directions

Given the different focal points of the four studies, several implications can be discussed. Although psychosocial stress improved concentration performance in our study, the results suggest that higher stress-induced cortisol increases may dampen this improvement. These findings have implications for practical application. Individuals requiring high levels of concentration, e.g., because of their professional occupation, may profit from HPA-axis

habituation to stress. Accordingly, previous research has demonstrated that the extent of HPA-axis activation triggered by a stressor is reduced with repeated exposure to that particular stressor (Grissom & Bhatnagar, 2009; Kothgassner et al., 2021). This habituation might counteract the observed negative effects of stress-induced cortisol on concentration performance. Moreover, as acute stress can shift cognitive control towards habitual processes (Schwabe, 2017; Vogel et al., 2016), practicing concentration performance strategies could also be beneficial with respect to the impairing effects of cortisol. Suggested strategies include focusing on factors within an individual's control and taking action, rather than dwelling on doubts, when feeling nervous (Moran, 2012).

Regarding eating after stress, our findings suggest that women could be more prone to obesity than men because of hormonal differences, with higher overall cortisol levels in men and men's CCK levels being reactive to stress and eating after stress. Cortisol and gut hormones could therefore be potential targets for the treatment of obesity (Troke et al., 2014). A deeper understanding of the regulation of appetite and satiety as well as their influencing factors (such as stress) may therefore be of special interest in the development of pharmacological strategies for patients with obesity (Alhabeeb et al., 2021; Troke et al., 2014).

The findings of the third and fourth study suggest that the stress reactivity of intermediate biological risk factors (such as blood lipids) and the dysregulation of the basal HPA-axis activity may represent early indicators for increased cardiovascular risk. Indeed, mechanisms underlying allostatic load may be interesting targets for the detection of high-risk individuals and therefore allowing earlier interventions (Juster et al., 2010). Accordingly, the monitoring of stress-reactive parameters and basal HPA-axis activity may help to identify high-risk individuals. The importance of basal blood lipid levels in predicting future cardiovascular risk has already been well established (e.g., Calling et al., 2021). It needs to be elucidated

whether the stress reactivity of intermediate biological risk factors for CHD, such as blood lipids, provides additional value for the identification of high-risk groups.

Nevertheless, our results emphasize the urgency of reducing stress in high-risk and patient groups, as it has already been proposed earlier (Rozanski et al., 2005). The effectiveness of stress management techniques to reduce atherosclerosis in hypertensives (Castillo-Richmond et al., 2000) and to improve medical outcomes in CHD-patients (Blumenthal et al., 2016) has already been proven. Future studies should also evaluate the efficacy of psycho-social interventions in reducing subclinical markers of allostatic load and intermediate biological CHD risk factors (Juster et al., 2010). To this end, it may be worth investigating whether positive psychological factors, such as optimism and positive affect (Levine et al., 2021) can have a buffering effect on intermediate biological CHD risk factors.

The findings of the present thesis support the assumption that the relationship between stress and health is multidimensional (e.g., Cohen et al., 2016), with direct and indirect effects of stress (O'Connor et al., 2021; Steptoe & Kivimäki, 2012). In accordance with previous evidence (McEwen & Seeman, 1999; O'Connor et al., 2021), the third and the fourth study indicated that stress influences health via mediators of the stress system and related biological systems. These effects were particularly important for hypertensives and CHD-patients. The results of the second study suggested that acute stress may affect health indirectly via effects on eating (at least in healthy women). These stress-induced changes are discussed as further risk factors for CHD (Hill et al., 2022; Tomiyama, 2019). The results of the thesis emphasize the need to investigate the relationship between stress and health at different levels in future studies. In healthy individuals, stress may impact health indirectly via health behaviors that promote disease risk in the long term. In contrast, in hypertensives and CHD-patients, stress directly contributes to changes in related biological systems that further increase their vulnerability to the beginning and gradual progression of atherosclerosis. Continued research

could determine acute stress effects on health behaviors in high-risk and diseased individuals and examine whether changes herein additionally contribute to their health risk in the long term.

Concluding Remarks

Stress has become a prevalent part of everyday life with multiple biological, cognitive, behavioral, and health-related effects (e.g., Chrousos, 1998; McEwen & Stellar, 1993). The present thesis attempted to capture the short-term and long-term effects of acute psychosocial stress and HPA-axis (re)activity. We found an enhanced concentration performance following acute psychosocial stress that was associated with lower stress-induced cortisol increases. With respect to the mechanisms of eating after stress, a lower immediate total cortisol stress reactivity was related to higher perceived hunger. As compared to men, women had lower overall cortisol and non-reactive CCK levels, pointing to a higher risk for stress-induced eating in women (e.g., Du et al., 2022). The results emphasized the need to consider sex-specific differences in the mechanisms behind stress effects. The thesis added further evidence for the association of stress (mediators) and CHD risk (e.g., Kivimäki et al., 2012; Rosengren et al., 2004; Steptoe & Kivimäki, 2012). Our findings pointed to an altered regulation of the stress system in hypertensives and CHD-patients, with a higher blood lipid stress reactivity in hypertensives and a lower basal HPA-axis activity in hypertensives and CHD-patients as compared to healthy normotensives. These observed dysregulations were associated with long-term CHD risk in terms of prospective increases in intermediate biological risk factors. Our findings support the assumption that stress and related allostatic changes play a vital role in the progression and outcome of CVD (Juster et al., 2010).

The thesis demonstrated that various approaches, such as including different study samples and employing cross-sectional and prospective study designs, are valuable in

comprehending the complex underlying mechanisms of stress. Despite, or perhaps because of the different methodological approaches and focal points of the studies included in the present thesis, many questions, especially about sex-related differences and contributing psychosocial mechanisms, have been raised that need to be addressed in future studies. At present time, the words of Selye (1950) remain valid: “It will take many years, indeed many generations, before the details of the general adaptation syndrome [stress] are satisfactorily elucidated. In fact, we shall never truly ´understand´ this phenomenon, since the complete comprehension of life is beyond the limits of the human mind” (p.1383).

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